QUARTERLY REVIEWS

THE TRIPLET STATE

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In the last fifteen years there has been a steady accumulation of experimental and theoretical evidence showing that long-lived excited states play an important rôle in determining not only luminescence behaviour but also the chemical reactivity of many molecules. The commonest kind of metastable excited state is one in which there are two unpaired electrons aligned with parallel spins, resulting in a so-called " triplet " state.

Because such triplet states—and states of other multiplicity—were first uncovered in gas-phase spectroscopic studies of atoms and diatomic molecules much of the terminology in common use is that of the spectroscopist rather than of the chemist, who for example is usually more familiar with the term " diradical " than " triplet state " for the description of a molecule with two unpaired electrons.

The object of this Review is therefore to clarify these concepts, to give a brief historical account of the development of our knowledge of this important subject, and to survey the more recent developments which are of interest to the chemists and biochemists rather than merely to the spectroscopist.

Spectroscopic origin of the triplet state

In the " ground " (*i.e.*, unexcited) state of most molecules each electron is paired with another—*i.e.* there are two electrons in each orbital. The Pauli principle then tells us that, since the quantum numbers determining their spatial configuration are identical, and they must differ in some respect, the spins of the two electrons are opposed, and cancel each other. The result is a diamagnetic molecule in which spin contributes nothing to the total angular momentum of the electrons. Such a state, for reasons which will become obvious later, is called a singlet state.

Let us suppose that one of the paired electrons is excited, by radiation or other means, to a different electronic energy level. Now the spatial quantum numbers of the two electrons are different, and the Pauli principle consequently makes no demand about the electron spin. There are two possibilities—spins opposed or spins parallel. The first is again a diamagnetic " singlet ", but in the second the effects of the spinning electrons are added instead of cancelling each other. The spinning electrons now contribute angular momentum to the system and also, as a result of their spinning charges, behave as small magnets which align themselves if an external magnetic field is applied and result in paramagnetism.

To obtain the total angular momentum (J) of the molecule, which determines where the corresponding energy level lies, we may use the vector model, and combine vectorially the total orbital angular momentum L and the total spin angular momentum S. In the case now considered, if $L > S$, this can be done in three ways (Fig. 1) in which the spin angular momentum is added to, subtracted from, or has no effect on the value of L. The rule for this "quantised" vector addition is $J = (L + S), (L + S - 1), \ldots, (L - S)$.

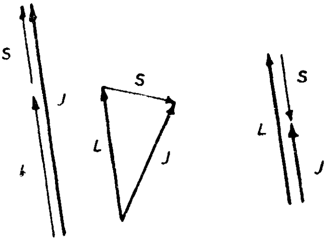

FIG. 1

Vector addition of $L = 2$ *and* $S = 1$ *to give three components of a triplet.*

Thus if the two unpaired electrons are p electrons, with resultant orbital angular momentum $= 2$ (D state), we have the three possibilities $^3D_3$, $^3D_2$, $^3D_1$, where the superscript denotes the multiplicity, and the subscript the value of J. The result is three slightly different energy levels. Of course only one is present in any given molecule, but in an actual spectroscopic measurement, when light is absorbed by or emitted from perhaps 10^{20} molecules, all three species are present. This is the origin of the term " triplet ", as compared with singlet states ($S = 0$), which for a given value of L can have only a single value of J. It is easily shown that the *multiplicity* (singlet, doublet, triplet, etc.) is given by the expression

$$M = 2S + 1$$

where $2S$ is the number of unpaired electrons.

If we are considering the gas-phase spectra of small molecules where the individual levels are clearly resolved, the multiplicity is easy to determine from spectral fine structure. But in the spectra of liquid or solid substances the resolution of individual lines is rare indeed (although possible, for instance with liquid hydrogen). Consequently to identify the multiplicity of the unresolved broad spectral bands, other techniques must be used, some of which will be mentioned subsequently.

What determines whether we obtain in the excited state a singlet or a triplet ? Application of conservation laws similar to the law of conservation of energy gives the answer. For molecules containing only light atoms (first-row elements) the spin angular momentum of the electrons can be considered as an independently quantised quantity, the value of which must be conserved during a transition brought about by radiation (L–S

coupling). Consequently if, as is usual, the ground state is a singlet, then so is the excited state. Otherwise angular momentum would have to appear from nowhere or to disappear. In quantum-mechanical formulation, we write

$$\Psi = U_{(\text{space})}S_{(\text{spin})}$$

where Ψ is the total wave function and U and S its spin and space components. The non-relativistic Hamiltonian contains no terms involving the spin, so that in evaluating the matrix element $\int \Psi_1 H' \Psi_2 \, \mathrm{d}\tau$, where H' represents the perturbation bringing about transitions, it is possible to factorise out the integral over spin space $\int S_1 S_2 \, \mathrm{d}S$. The spin functions S_1 and S_2 are orthogonal, so that the integral, and consequently the transition probability between the states under consideration, is zero unless the two states have the same spin function, *i.e.* are of the same multiplicity.

Unfortunately, this approach, which would lead to the conclusion that all the observed levels of a given molecule should have the same multiplicity as the ground state, is only an approximation. For molecules containing only light atoms this approximation is a very good one in the absence of perturbing influences, but may be upset by the magnetic effect of heavy atoms, or atoms with unpaired electrons in the immediate environment, or by strong, inhomogeneous magnetic fields. Also the spin conservation rule applies to transitions involving radiation and cannot be applied without modification to " radiationless " transitions of the kind which will be discussed later. As molecules involving heavier and heavier atoms are considered, the increasingly inhomogeneous field of the heavy nucleus makes the rule worse and worse. Spin energy is no longer conserved because of the ready interconversion of spin and orbital momentum, only the total angular momentum j of the individual electrons obeying the quantum rules.

Consequently from the simple situation in which transitions with spin conservation are " allowed ", and those in which spin conservation would be violated are " forbidden ", we pass to a situation in which the " forbidden " transitions actually occur although naturally with lower probability than do allowed transitions. The " degree of forbiddenness " of singlet–triplet transitions is something about which we now have a good deal of experimental information. Various other factors besides the rule of conservation of spin may contribute to make a transition forbidden (low spatial overlap between the two states, cancellation of probabilities because of symmetry within the molecule, etc.), but for organic molecules without heavy elements, the restriction of spin conservation is the most severe, so that singlet–triplet transitions are the weakest ones observed.

Any of three quantities may be used to estimate the probability of a given transition. In emission work it is most usual to use the lifetime, since for emission-intensity measurements to be very useful self-absorption must be avoided, and a precise knowledge of the number of quanta absorbed is also required. Very few reliable experiments have been made of absolute quantum yield for emission (quanta emitted/quanta absorbed) in the condensed phase, although pioneering work has been done by Vavilov,[1] Bowen,[2] McClure,[3] and others.

In absorption, the integrated molar extinction coefficient

$$\int_{-\infty}^{\infty} \varepsilon(\bar{\nu})\, d\bar{\nu}$$

where the integration is obtained from the area of the extinction curve for the transition plotted with wave number $\bar{\nu}$ as abscissa, is a better measure of intensity than peak height at maximum absorption, although the latter is often used in qualitative work. Linearly related to the integrated extinction coefficient is the oscillator strength. This quantity measures the intensity of a given transition compared with that which would be observed for an allowed transition at the same frequency for a three-dimensional harmonic oscillator, for which an exact intensity calculation is possible. The final relationship is

$$f = 4{\cdot}33 \times 10^{-9} \int \varepsilon(\bar{\nu})\, d\bar{\nu}. \quad . \quad . \quad . \quad (1)$$

where f is the oscillator strength. For allowed transitions in actual molecules we usually find values of f to be a little less than unity, although values higher than unity may appear if several electrons in the molecule contributed to the intensity of the same transition. Since absorption bands usually have a half-width (*i.e.* width of the absorption band at half the maximum extinction coefficient) of a few thousand wave numbers, we see from eqn. (1) that for an allowed transition the maximum extinction coefficient likely to be observed is about 10^5. This value corresponds to a natural lifetime of about 10^{-9} sec., and deviations from these values (oscillator-strength unity, molar-extinction coefficient 10^5, or lifetime 10^{-9} sec.) may be used as a measure of forbiddenness.

For the usual kind of organic molecule without heavy elements, the triplet state lifetime is about 10^{-3} sec., corresponding to a molar extinction coefficient of 0·1, or an oscillator strength of 10^{-5} to 10^{-6}, *i.e.* the singlet–triplet transition probability is a little more than a millionth of that for the corresponding singlet–singlet.

Observation of the triplet state. Historical

Because of the low extinction coefficient of singlet–triplet transitions, they were not observed in absorption until special efforts were made to detect them. However, from a fairly early date long-lived (*i.e.*, persisting after the exciting source is removed) emission of light, or phosphorescence, was observed from irradiated molecules dissolved in " glassy " solvents. This technique was first introduced by Schmidt[4] in 1896 and was used

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[1] Vavilov, *Z. Physik*, 1924, **22**, 266.

[2] Bowen, *Trans. Faraday Soc.*, 1939, **35**, 44.

[3] Gilmore, Gibson, and McClure, *J. Chem. Phys.*, 1952, **20**, 829.

[4] Schmidt, *Ann. Physik*, 1896, **58**, 103.

extensively almost thirty years ago [5–7] by Vavilov and others, who showed that in most cases the decay of luminescence followed a first-order law, and was consequently not due to an ionisation-recombination process. In the last decade or so, principally as a result of the pioneering work of Lewis and his students in America, and of Terenin in the U.S.S.R., on the emission spectroscopy of complex molecules, the importance of excited triplet states, even in light-atom molecules, has been recognised. This area of spectroscopy is recent enough and important enough to warrant a brief historical account of its development being given.

In general, solid substances at room temperature do not show phosphorescence (delayed light emission) and only a small number show fluorescence. Since absorption experiments show clearly that the molecules are excited, it follows that radiationless deactivation processes can compete so successfully with the radiative mechanism that few molecules stay excited long enough to radiate. The faster the emission process, the greater the chance of its occurrence; hence the rareness of the slow phosphorescence process which allows ample time for competing processes, compared with fluorescence.

In solution the same is true. Deactivation by collisions usually overwhelms and obliterates phosphorescence, although fluorescence occurs fairly frequently. If we prevent collisions by dissolving the molecule in a solvent which sets to a rigid glass on cooling (or even if we " dissolve " it as an impurity in the crystal of another substance) the competing processes are considerably reduced. Many substances so dissolved are fluorescent, and a few are phosphorescent even at room temperature (*e.g.* fluorescein in a boric acid glass).

Thermal agitation still disturbs the molecule so much that radiationless deactivation can occur, however. If we cool the system strongly, the perturbation is much reduced. At high dilution and a temperature below 90° K almost all substances are fluorescent, except those with very efficient " internal " quenching processes, and a great many are phosphorescent. Under these conditions, then, the emission of light becomes a general phenomenon, which can be investigated systematically.

In 1935 Jablonski [8] proposed a scheme to explain fluorescence and phosphorescence spectra in terms of excitation to a short-lived state, in which the molecule could either emit fluorescence or switch to a different " metastable " excited state. Phosphorescence is then produced during the delayed return from the metastable state to the normal ground state. Except that he did not recognise the " metastable " phosphorescent state as the triplet state, Jablonski's interpretation is essentially that currently in use (Fig. 2). Lewis, Lipkin, and Magel [9] showed that the phosphorescent state could be characterised by an absorption spectrum different from that of the parent molecule. They used fluorescein dissolved in boric acid and

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[5] Vavilov and Lewschin, *Z. Physik*, 1926, **35**, 920.
[6] Schilschlowski and Vavilov, *Phys. Z. Sowjet.*, 1934, **5**, 379.
[7] Lewschin and Vinokurov, *ibid.*, 1936, **10**, 10.
[8] Jablonski, *Z. Physik*, 1935, **94**, 38.
[9] Lewis, Lipkin, and Magel, *J. Amer. Chem. Soc.*, 1941, **63**, 3005.

were able to excite more than 80% of the fluorescein molecules into the triplet state by means of a high-pressure mercury arc and to determine the resulting change in absorption (Fig. 3). These authors speculated that the metastable state might be a triplet, but were unable to decide between this and a true tautomerism involving a change in the arrangement of nuclei, the identification of this state being made possible by its preservation in the rigid medium. Lewis and Kasha,[10] basing their argument on the existence of two sets of levels between which transitions appear to be strongly forbidden for many molecules, and on considerations of the changed conjugation brought about in the triplet state, decided that definite identification of the phosphorescent state with the triplet level could be made. Next, Lewis and Calvin [11] reported the occurrence of paramagnetism in the phosphorescent state, thus confirming in a rather difficult experiment the existence of unpaired electrons.

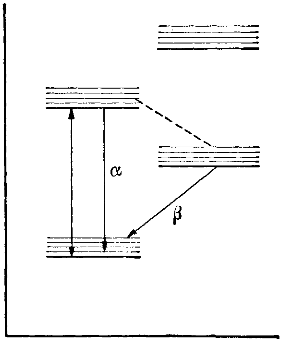

FIG. 2
Jablonski diagram for phosphorescence emission.

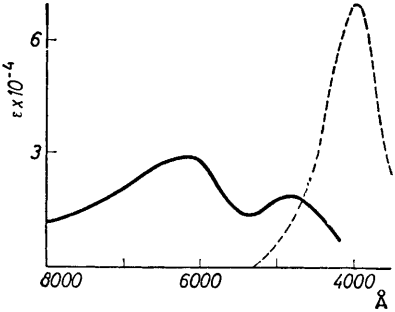

FIG. 3
New absorption spectrum (solid line) appearing (reversibly) on irradiation of fluorescein in boric acid glass. The dotted line shows the normal absorption spectrum. (After Lewis, Lipkin, and Magel.[9])

[10] Lewis and Kasha, *J. Amer. Chem. Soc.*, 1944, **66**, 2100.
[11] Lewis and Calvin, *ibid.*, 1945, **67**, 1232.

Perhaps the most conclusive evidence, showing that in almost every example studied, the forbiddenness of the transition from the phosphorescent state is due to the spin-conservation rule, comes from the work of McClure.[12] As we have remarked, singlet–triplet transitions occur with quite high probability in the spectra of heavy atoms, under the influence of the high field gradient. (This is as a consequence of singlet–triplet mixing, all singlet states containing some triplet character, and *vice versa.*) Using atomic data to estimate spin-orbit coupling, with the assumption that in a molecule the contribution of the heaviest atom near a given electron will be overwhelmingly the most important, McClure obtained the following estimate of triplet-state lifetimes for molecules with the atom named as the heaviest component: carbon 0·003 sec., nitrogen 0·0004 sec., oxygen 0·0001 sec. By observing series of organic molecules with different halogen or heavy-metal substituents, he was also able to show that the relative lifetimes were approximately in the ratio to be expected from the spin-orbit coupling data already known for the heavy atom.

The calculations are approximate and among other things do not take account of cancellations of some of the contributions to spin-orbit coupling which may occur as a result of molecular symmetry. In particular, large anomalies occur between the predicted (0·003 sec.) and observed (several seconds) lifetimes of aromatic hydrocarbons. More refined calculations by McClure and by Mizushima and Koide [13] show that this is satisfactorily accounted for when the symmetry of the orbitals concerned is considered. Kasha [14] extended the " heavy-atom " technique for increasing transition probability to the case where the heavy atom is supplied by the solvent. It was shown that the singlet–triplet absorption of 1-chloronaphthalene, too weak for observation in most solvents, was clearly observed ($\varepsilon \simeq 0{\cdot}01$) in ethyl iodide. The careful researches of Kasha and his co-workers have indicated other cases of heavy-atom effects brought about by alkyl iodide solvents. It must be emphasised, however, that because of the lability of the carbon–iodine bond, this technique is fraught with difficulties, and the possibility that the absorbing molecule may have been iodinated must be guarded against.

McClure's and Kasha's work, and subsequent contributions by other authors, leaves little doubt that most phosphorescence results from a transition involving a change in multiplicity, usually between singlet and triplet states. An exhaustive survey of the lifetimes of substituted aromatic compounds, in particular benzene derivatives, is given by Dikun *et al.*,[15] and new, very accurate data have come from Terenin's [16] laboratory on the lifetimes of excited pigment molecules.

A well-authenticated observation of the heavy-atom effect is found in the triplet-state lifetimes of a series of metal derivatives of dibenzoylmethane

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[12] McClure, *J. Chem. Phys.*, 1949, **17**, 905.

[13] *Idem, ibid.*, 1952, **20**, 682 ; Mizushima and Koide, *ibid.*, p. 765.

[14] Kasha, *ibid.*, p. 71.

[15] Dikun, Petrov, and Sveshnikov, *J. Exp. Theor. Phys.*, 1950, **21**, 63.

[16] Dmitrievski, Ermolaev, and Terenin, *Doklady Akad. Nauk*, 1957, **114**, 751.

examined by Yusta and Weissman.[17] Their results, presented in Table 1, are intriguing in that they do not show the simple dependence on atomic number which might be expected if the ligand orbitals were equally well coupled to the metal in every case. The ion size and magnetic properties would appear to be important factors in determining the amount of coupling. The decrease in triplet-state lifetime is accompanied by a decrease in the ratio Q (fluorescence)/Q (phosphorescence) (where Q = quantum yield). Interpretation of this result is rendered difficult in that the increasing phosphorescence might arise because of the increasing influence of S–T conversion, or because the triplet state, persisting for less time, is less susceptible to solvent quenching.

TABLE 1

Metal	Al	Sc	Y	Lu	Gd	La
Lifetime	0·5	0·3	0·24	0·12	0·002	0·09

Quite efficient singlet-triplet conversion has recently been demonstrated in porphyrin-like molecules by Kasha and Becker.[18] The fluorescence ($\tau < 10^{-4}$ sec.) and phosphorescence ($\tau > 10^{-4}$ sec.) of a number of ætioporphyrins, phthalocyanines, and phæophorbides were examined. It was found that the molecules with no paramagnetic metal ion (Mg^{2+}, Zn^{2+} compounds) showed strong fluorescence, but that paramagnetic Cu^{2+} and Ni^{2+} compounds showed phosphorescence, but no fluorescence. There is little doubt that one of the most significant differences between the two most widely distributed porphyrins, chlorophyll and hæm, arises from the fact that the magnesium ion is diamagnetic, while that of iron is paramagnetic and facilitates singlet–triplet conversion.

Position of triplet levels

The absorption and the emission spectra of a typical molecule showing both phosphorescence and fluorescence are illustrated in Fig. 4*a*. The mirror-image relation between absorption and emission arises because absorption is from the zero-point vibrational level of the ground state, to vibrationally excited levels in the upper state, and consequently requires more than the 0–0 energy, while emission is from the zero-point vibrational level in the excited state to vibrationally excited levels in the ground state, and consequently occurs with emission of less than the 0–0 energy. These two facts are a consequence of the Frank–Condon principle which tells us that " vertical " transitions are most probable, and of the fact that, although vibrationally excited molecules are produced in the upper electronic state, they lose their vibrational energy before they re-emit (Fig. 4*b*). Fig. 4*b* also shows how the radiationless transition to the triplet state occurs at the crossing point, where the excited singlet and triplet states have both the

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[17] Yusta and Weissman, *J. Chem. Phys.*, 1949, **17**, 1182.
[18] Kasha and Becker, *J. Amer. Chem. Soc.*, 1955, **77**, 3669.

same energy and co-ordinates. A mirror-image relation is not shown for triplet absorption and emission, because normally triplet absorption is not observed. In the special experiments where triplet absorption has been detected, the mirror-image relation is again apparent.

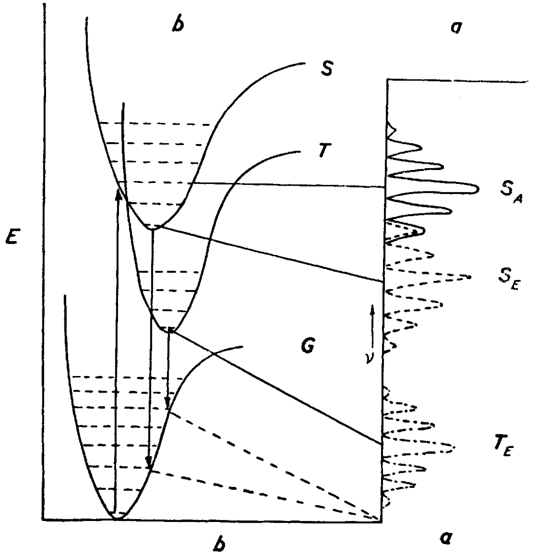

FIG. 4

(*a*) *The low-energy absorption* (S_A) *and emission* (S_E *and* T_E) *spectra of a typical molecule.* (*b*) *Potential curve showing how these spectra arise* (G = *ground state*).

It will be seen that in Fig. 4 we have placed the lowest triplet level as the lowest excited state of the molecule. This is in accordance with Hund's rule, that if both singlet and triplet configurations are possible with the same set of orbitals the triplet level will be at lower energy than the corresponding singlet. The necessity for an antisymmetric total wave function precludes the existence of a triplet ground state for most molecules since the triplet spin function is symmetric and must be combined with an antisymmetric space function, whereas most molecules have paired electrons and therefore a symmetric space function in the ground state. The lowest triplet state is found experimentally to be usually the one responsible for phosphorescence emission, whereas fluorescence occurs from the lowest excited singlet state to the ground state which is, of course, the lowest singlet state. Thus we find naphthalene with fluorescence in the ultraviolet but phosphorescence in the green region of the spectrum.

Since, until irradiation of sufficiently high energy is used for gross decomposition of the molecule, the emitted radiation comes from one or both of these two states, regardless of what state is excited by the incoming radiation, the molecule must undergo radiationless transitions from the initially excited to successively lower electronic levels until it finally makes the radiative jump to ground. It may reasonably be asked why only the last step is a radiative one. The answer is that the largest energy gap is usually between the ground and the first excited state, the density of states increasing with increasing energy, so that the necessary overlapping of potential-energy surfaces required for a radiationless transition is less likely between these two than between closer lying pairs. There are many cases of substances non-fluorescent even under the most favourable conditions, however, and for many of these, radiationless transitions to the ground state must be assumed. Furthermore, a few cases of emission from states other than the lowest-excited ones are known and have been recently extended by the investigations of Pesteil *et al.*[19] at very low temperatures, which suggest that the radiationless transitions are much reduced or inhibited at 4° C and may therefore be facilitated by phonon perturbations (and possibly by rotational effects) when they occur in systems at 80° K, where very few if any vibrational modes can be excited.

Most studies of the triplet state have been focused so far on aromatic or other highly conjugated molecules, although some investigations have been made also on aliphatic aldehydes, ketones, and a few miscellaneous substances. For many of these substances it has been possible to identify the pair of levels, one triplet and one singlet, which are associated with each other, *i.e.* have the same species designation except for singlet or triplet character. The separation between the pair is usually between 8000 and 13,000 cm.$^{-1}$ (about 1—1·6 ev) and the observed value compares quite well with theoretical estimates in the case of a number of aromatic hydrocarbons where explicit calculations have been made (Table 2).

TABLE 2. *Comparison of energies* (ev) *of related singlet and triplet states**

Molecule	Singlet			Triplet		
	designation†	energy		designation†	energy	
		calc.	obs.		calc.	obs.
Benzene	${}^1B_{2u}-$	4·710	4·71	${}^3B_{2u}-$	4·71	—
	${}^1B_{1u}+$	5·96	5·96	${}^3B_{1u}+$	3·59	3·59
	${}^1E_{1u}+$	6·548	6·76	${}^3E_{1u}+$	4·149	—
Naphthalene	${}^1B_{2u}+$	4·493	4·38	${}^3B_{2u}+$	2·18	2·64
Anthracene	${}^1B_{2u}+$	3·648	3·31	${}^3B_{2u}+$	1·66	1·85

* Calculated values from Pariser.[20]
† See texts on group theory for explanation of labelling.

From the easily observable position of the lowest-excited singlet state, *i.e.* the lowest energy at which the molecule shows appreciable absorption of light, it is therefore possible to predict roughly where the corresponding triplet emission should be. This is often important, since the triplet emission may be faint and in a region of the spectrum only accessible by the use

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[19] Pesteil, Zmerli, and Pesteil, *Compt. rend.*, 1955, **241**, 29.
[20] Pariser, *J. Chem. Phys.*, 1956, **24**, 250, and references therein.

of specially sensitised photographic plates. Because of energy-transport phenomena, strong luminescence may result from small amounts of impurities with low-lying energy levels, and the observation of emission spectra is fraught with difficulties. Already cases have been recorded of triplet emissions claimed for molecules which subsequently proved to be due to impurities. The latter, although present in quantities less than 1 in 10^6, may nevertheless be responsible for the whole of the emission observed. Extreme care in the preparation of samples is therefore necessary and, where possible, confirmatory absorption experiments should be attempted. True singlet–triplet transitions usually have an extinction coefficient which allows them to be detected in fairly long-path absorption experiments with concentrated solutions, but spurious " impurity " emissions of equal intensity are often completely undetectable in absorption.

Higher triplet levels

The passage from a singlet to a triplet state is a highly forbidden one, but once we attain the triplet condition, a whole series of more highly excited triplet levels are readily accessible. The pioneering work for organic molecules is that of Lewis, Lipkin, and Magel,[9] who found that the absorption of a sample of fluorescein in a boric acid glass was radically changed if the measurement was made while the sample was illuminated by a high-intensity mercury arc. The high-intensity source populated the lowest triplet level sufficiently highly for triplet–triplet absorption to occur under the influence of the measuring beam.

With ordinary light sources, this kind of measurement is possible only in rather favourable circumstances.[21] However, the development of " flash " sources in which a high-intensity spark is discharged very rapidly through a circuit with very large capacitance has led to the observation of many new cases of triplet–triplet absorption. Porter and Windsor [22] have been able to detect the triplet–triplet absorption spectrum for several molecules with long-lived triplet states in liquid solutions (usually hexane) at room temperature. This represents a considerable step from earlier triplet-state investigations which were effectively restricted to solid solutions. It opens the possibility of studying the kinetics of triplet-state disappearance in varying environments and hence essentially the chemical properties of the triplet state. A step in this direction has been taken by Terenin *et al.* with metal phthalocyanines.[23]

A recent development in the observation of triplet–triplet absorption by flash technique has been the application to chlorophyll.[24, 25] There has been in the past considerable debate about the possible triplet levels of this substance. The difficulties are largely associated with the fact that the

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[21] McClure, *J. Chem. Phys.*, 1951, **19**, 670.

[22] Porter and Windsor, *ibid.*, 1953, **21**, 2088.

[23] Terenin, Karyakin, Lubomudrov, Dmitrievski, and Sushinski, *J. Opt. Spekt.*, 1956, **1**, 456.

[24] Livingston, *J. Amer. Chem. Soc.*, 1955, **77**, 2179.

[25] Livingston, Porter, and Windsor, *Nature*, 1954, **173**, 485.

triplet-state phosphorescence should lie in the infrared region where effects from impurities are most troublesome, and that it is difficult to obtain either of the chlorophylls chemically pure. The flash experiments of Livingston *et al.* show quite unequivocally that irradiation of both chlorophyll-a and chlorophyll-b results in the formation of a metastable excited state. Characteristic new triplet–triplet absorption bands were observed at about 460 mμ for chlorophyll-a, and 485 mμ for chlorophyll-b, both decaying with a half-life of a few ten-thousandths of a second (Fig. 5).

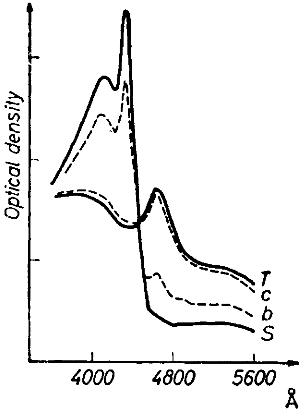

FIG. 5

Triplet–triplet absorption by chlorophyll-a. Curve S, normal absorption. Curves b and c are taken closer and closer to the time of the exciting flash. Curve T is extrapolated to zero time and represents the triplet–triplet absorption band. (After Livingston.[24])

With chlorophyll-b, the disappearance rate is linearly dependent on the total concentration used in the experiment. This suggests that radiationless quenching occurs only when an excited molecule meets another pigment molecule, and not by non-specific solvent quenching. This observation, like that by Calvin and Dorough on zinc chlorin mentioned later, suggests that in the large porphyrin-type molecules, triplet-state quenching is a comparatively difficult and specific process. This is in contrast to singlet-state quenching for these substances, and in contrast to the ready triplet-state quenching found in most simpler molecules. It may be that the unpaired electrons are rather well localised on nitrogen atoms near the middle of the molecule and are protected against deactivation by random collisions.

cis–trans-Isomerisation

In ethylenic compounds, *cis–trans*-isomerisation about the double bond can proceed *via* the triplet state of the molecule. Irradiation with ultraviolet light usually brings about the conversion of the stable *trans*-isomer into an equilibrium mixture of the *cis*- and the *trans*-form. The energy of activation for the reversion of the *cis*- to the *trans*-form varies from about 15 to 50 kcal. for different compounds. Consequently, it is possible to study the kinetics of the spontaneous *cis–trans*-conversion at reasonably low temperatures (100—400° C).

The reaction rate for this unimolecular process may be written

$$k = Ae^{-E/RT}$$

Experimentally, it is possible to assign values to the quantities A and E, and it is observed that the results fall into two well-defined groups:[26]

(1) A group for which the activation energy E is low (*ca.* 20 kcal.) but the frequency factor A, which is a measure of the number of molecules satisfactorily endowed with energy which actually isomerise, is also abnormally low (about 10^4 sec.$^{-1}$).

(2) A group for which the activation energy is almost twice as large, but for which the frequency factor is much more in line with the value found in most reactions (about 10^{11} sec.$^{-1}$).

These results are particularly interesting in view of considerations put forward many years ago by Mulliken[27] about the excited states of ethylene and ethylene-like molecules. The simplest excitation process is for an electron to jump from the π (double bond) orbital to the corresponding antibonding level. Calling the carbon atoms C_1 and C_2 we write this

$$(2p_{C_1} + 2p_{C_2})^2 \longrightarrow (2p_{C_1} + 2p_{C_2})^1(2p_{C_1} - 2p_{C_2})^1$$

The results of this are two-fold. First, the wave function, exclusive of spin, is now antisymmetric (because of the single electron in the antisymmetric antibonding orbital) and, as a consequence of the Pauli principle, the spin function must be symmetric. We can write the three possible spin functions as

$$\begin{array}{l} \alpha_1\alpha_2 \\ \beta_1\beta_2 \\ \alpha_1\beta_2 + \beta_1\alpha_2 \end{array}$$

Second, the destabilising effect of the antibonding electron outweighs somewhat the bonding energy of the electron left behind in the bonding orbital. Consequently, the ground-state nuclear configuration, which put the π orbitals parallel to each other to maximise their overlap, is now no longer the most stable configuration. By twisting the molecule through 90° about the C–C axis, the overlap in both the π-bonding and π-antibonding orbitals is minimised, and the most stable configuration consequently attained. The plot of the potential-energy situation for the ground and lowest excited triplet state is consequently as shown in Fig. 6, from which it becomes clear that as the molecule is twisted out of plane the triplet state becomes more stable than the singlet, and indeed, for the 90°-twisted configuration, should be considered the ground state (just as the ground state of the oxygen molecule, with which ethylene is isoelectronic, is a triplet). Of course, in substituted ethylenes, where the *trans*-form is more stable than the *cis*, the curves will not be exactly symmetrical.

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[26] Glasstone, Laidler, and Eyring, " The Theory of Rate Processes ", McGraw-Hill, New York, 1941.

[27] Mulliken, *Phys. Rev.*, 1932, **41**, 1751.

We thus see that in carrying out the 180° twist necessary for the *cis–trans*-isomerisation, two pathways are possible. We might follow the minimum-energy path, passing at point I to the triplet and at point II back to the singlet state. However, because of the spin-conservation rule, and the fact that the energy levels in states I and II may not match exactly, the number of fruitful collisions is lower than in the case of a simple potential barrier of the same height; *i.e.* the reaction is slowed because of the difficulty of the *S–T* and *T–S* transitions. This pathway is clearly that followed by the group of substances having low activation energy and low frequency factor, while the second possibility, that the molecule stays permanently in the singlet state and goes over the high barrier, corresponds to the second group, with a normal frequency factor and higher energy of activation.

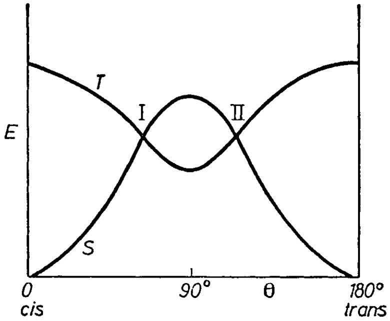

FIG. 6

Potential curves for twisting ethylene-like molecules about the double bond through an angle θ. S, singlet ground state. T, excited triplet state.

The question of what determines the actual pathway followed has not yet been satisfactorily settled. If we examine the molecules which fall into the two groups we find that the triplet-state pathway is followed by molecules with aliphatic groups (apart from the single ethylene bond), whereas the presence of aromatic substituents favours the singlet-state pathway. This may be explicable by the fact that when it is part of a conjugated system, the double-bond character of the ethylenic bond is decreased and the potential barrier to twisting in the singlet state is reduced. It is, in fact, true that in spite of the different parameters in the rate-constant expression for the two groups, the overall reaction rates in the temperature range 250—350° c, the most exhaustively studied region, are not very different from each other.

Unfortunately, the exact locations of the triplet levels of simple ethylenic molecules have not been determined experimentally, and as yet no calculations have been made accurately enough for meaningful comparison with the activation energy found for the low-barrier reactions. Most estimates suggest a value considerably higher than 20 kcal. above the ground value for the triplet state. Early semi-empirical calculations based on a twisting frequency of 825 cm.$^{-1}$ gave reasonable agreement for the activation energy for the singlet-state pathway, but, in view of more recent work on ethylene, can hardly be considered reliable. Measurements of reaction rate as a function of pressure are quite consistent with the triplet mechanism for the reaction *via* the pathway of low activation energy. It is also significant that the fall in frequency factor (*ca.* 10^6) is of the same order of magnitude as the reduction in transition probability of singlet–triplet compared with singlet–singlet transitions observed spectroscopically.

It is interesting confirmatory evidence for the low frequency factor in isomerisations being connected with the special properties of the excited state of the ethylenic π orbital that the frequency factor is quite normal ($\simeq 10^{13}$) for the *cis–trans*-isomerisation of azobenzene, which process apparently occurs exclusively *via* the singlet process with an activation energy of about 23 kcal.[28] The barrier is presumably lowered by the interaction of the π electron of one nitrogen atom with the unshared pair of the other in the twisted position.

More recent work on *cis–trans*-isomerisation [29] has extended Kistiakowsky's conclusions [30] to reactions in solution (most of the earlier work was concerned with the gas phase). Of the isomerisations examined, those of maleic acid and dimethyl maleate followed the low-energy (triplet) path, and those of cinnamic acid and various cinnamic esters went *via* the singlet. The generalisation that ethylenes with aromatic substituents can follow the singlet path is thus upheld, and no cases of intermediate energies of activation or frequency factors between the two extremes have been reported.

Chemical reactions involving the triplet state

As early as 1927 Perrin and his co-workers [31, 32] showed that various antioxidants (potassium iodide, phenols, aminophenols, thiourea, phthalimide, uric acid, etc.) have a strong fluorescence-quenching influence, and Perrin sought to associate this with a paramagnetic-quenching process. In a surprisingly well-informed work for its date, Mlle. Boudin,[33] in Perrin's laboratory, distinguished between the short-lived fluorescence of eosin and a longer-lived phosphorescence with a lifetime of about 10^{-3} sec. She showed that the apparent lifetimes of both fluorescence and phosphorescence were decreased by the addition of potassium iodide, but that the phosphorescence curve showed a closer relation to that portraying the inhibiting effect of potassium iodide on photo-oxidation. The conclusion was that the photo-oxidation proceeded *via* the metastable state, which was not at that time identified with the triplet.

A recent study has been made of the photoreduction of eosin Y with allylthiourea,[34] a molecule of great interest because of its use in many biochemical reductions. Allylthiourea brings about the reduction of excited eosin molecules without causing an appreciable drop in the fluorescence

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[28] Halpern, Brady, and Winkler, *Canad. J. Res.*, 1950, **28***B*, 140.

[29] Davies and Evans, *Trans. Faraday Soc.*, 1955, **51**, 1506.

[30] Kistiakowsky and Smith, *J. Amer. Chem. Soc.*, 1936, **58**, 2428, and earlier papers.

[31] Perrin, *Compt. rend.*, 1927, **184**, 1097.

[32] *Idem, ibid.*, p. 1121.

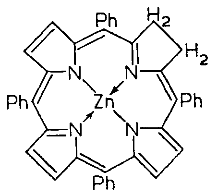
[33] Boudin, *J. Chim. phys.*, 1930, **27**, 284.

[34] Oster and Adelman, *J. Amer. Chem. Soc.*, 1956, **78**, 913.

intensity. This fact, the observed rather low quantum yield (max. = 0·092), and the observed kinetics indicate that allylthiourea reacts with a metastable species which is derived from, but not reconverted into, the fluorescent (singlet) state. The observation of a decreasing quantum yield with increasing dye concentration, even at comparatively low molarity (10^{-6}M) where a collision between two dye molecules can only be expected after about 10^{-4} to 10^{-5} sec., confirms again that the metastable state has a lifetime in the range to be expected for the triplet state.

Triplet States in Porphyrin-like Molecules.—Because of the part which must be played by an excited state of chlorophyll in the interaction of photosynthesis, much attention has been paid to the way in which chlorophylls and porphyrin-type molecules in general can react, and several results suggest that the triplet state may be an important intermediate.

An early investigation by Calvin and Dorough [35] on the photo-oxidation of zinc chlorin to the corresponding porphin (removal of two hydrogen atoms from the reduced pyrrole ring) by β-naphthaquinone, showed the reaction to be of zero order with respect to the quinone, and to occur with a quantum efficiency of only a few percent, but with no side reactions. This result is remarkable, inasmuch as that, if the quinone had to compete with the deactivating processes for excited zinc chlorin molecules, the reaction should certainly depend on its concentration. The result can be explained in one of two ways. First, if deactivation as well as reaction was brought about only by the quinone. In view of the usually severe solvent quenching of excited states in solution, this seems unlikely. The second alternative is that the primary step involves the excited molecule's passing by a radiationless transition into a metastable (presumably triplet) state, and that once it arrives in this state it is always oxidised. Quenching effects occur only before this reacting species is formed, and consequently the naphthaquinone does not have to compete for the molecules it oxidises.



If this interpretation, which was favoured by Calvin and Dorough, is correct, this is a true instance of reaction in the triplet state. It is still a rather remarkable case, since the triplet states of most molecules show strong collisional quenching at room temperature. However, it does appear that a triplet state is involved.

Huennekens and Calvin [36] have extended this work to other quinones. They find the quantum yields proportional to the oxidation potential of the used (indicating a higher proportion of successful collisions for the more easily reduced quinones) with a relation for *o*-quinones different from that for the *para*-series, the former being relatively more efficient (Fig. 7). This is understandable, if it is assumed that *o*-quinones can remove both hydrogen atoms in a single collision, whereas *p*-quinones cannot. They find that molecular oxygen fits reasonably well into the series of *o*-quinones if it is assumed that it is reduced to hydrogen peroxide by zinc chlorin (in contra-

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[35] Calvin and Dorough, *J. Amer. Chem. Soc.*, 1948, **70,** 699.

[36] Huennekens and Calvin, *ibid.*, 1949, **71,** 4024, 4031.

diction to a report in earlier work that oxygen inhibited the reaction). The rate of reaction was independent of quinone concentration over a concentration range 10^{-3}—10^{-6}M for a chlorin concentration of 10^{-5}M, but was dependent on the particular quinone used. These two facts could only be reconciled by recourse to the idea that, as well as providing the oxidant, the quinone is also the principal deactivating agent in the system, and that a long-lived intermediate is involved.

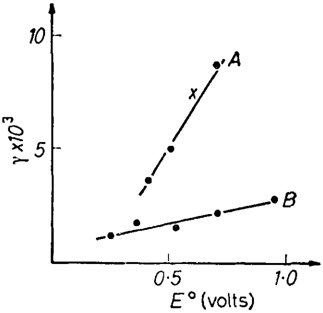

FIG. 7

Plot of quantum yield for the oxidation of zinc chlorin by various quinones : A, o-*quinones ; B,* p-*quinones ; x, oxygen.* (After Huennekens and Calvin.[36])

If the triplet state is indeed a necessary intermediate in the oxidation of the chlorin to the porphin it seems reasonable that it might also play a part in the reverse photoreduction of porphin to chlorin. With this in mind, Seely and Calvin [37] investigated the action of light plus a number of reducing agents (benzoin, *p*-dimethylaminobenzoin, ascorbic acid, dihydroxymaleic acid, hydrazobenzene, heptanethiol, etc.) on zinc porphin. Of these, all except benzoin reacted only slowly, whereas the last reduced the porphin, under suitable illumination, within 20 minutes.

If a similar mechanism to that in the oxidation prevailed, *i.e.* if all excited porphin molecules which reached the triplet state were reduced by the benzoin, a reaction showing zero-order dependence on benzoin and first-order dependence on light intensity might be expected. In fact, this is not the case. The rate is described by the expression

$$R = aF_{\text{b}} + bF_{\text{b}}F_{\text{p}}$$

where F_{b} represents the rate of light absorption by benzoin and F_{p} by porphin. This indicates that unexcited porphin molecules can be reduced by excited benzoin molecules. Indeed, the fact that for a given light intensity the rate is independent of porphin concentration means that excited benzoin molecules are almost sure to find a porphin molecule before deactivation. However, the constant b is much larger than a, the reduction being much more efficient if both molecules are excited. The authors believe that the excited benzoin molecule reacts in the triplet state, so that we have yet another example of the resistance of the triplet state to solvent quenching,

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[37] Seely and Calvin, *J. Chem. Phys.*, 1955, **23,** 1068.

this time in a smaller molecule. The presence of dissolved oxygen inhibits the reduction to chlorin. Instead, a degradative oxidation occurs, which the authors believe results from all the triplet-state benzoin molecules rapidly adding on an oxygen molecule, and the resulting complex oxidising the porphin with high efficiency.

The work of Krasnovsky and his co-workers[38] makes it almost certain that the photoreduction of all porphyrin-type molecules and phthalocyanines goes *via* the triplet state. They found that a number of substances quenching the fluorescence (usually oxidising agents such as *p*-benzoquinone) showed no significant amount of chemical reaction with chlorophyll, but that reducing agents such as ascorbic acid react reversibly with chlorophyll, but do not affect the (singlet state) fluorescence when they do so.

Later work by the same school [39] suggests that the reversible photoreaction of chlorophyll probably goes through the following stages :

$$\begin{array}{ccccc} \mathrm{X} & \rightarrow & \mathrm{X}^* & \rightarrow & \cdot\mathrm{X}\cdot \\ \cdot\mathrm{X}\cdot\ + & & \mathrm{AH} & \rightarrow & [\cdot\mathrm{XHA}\cdot] \\ & & & & \downarrow \\ \cdot\mathrm{XH}\ + & & \mathrm{A}\cdot & \leftarrow & \cdot\mathrm{X}^- + \mathrm{HA}^+ \end{array}$$

then $$\cdot\mathrm{XH} \xrightarrow{\text{similarly}} \mathrm{XH}_2$$

($\cdot\mathrm{X}\cdot$ denotes the triplet state)

The work on triplet emission of porphyrins by Kasha *et al.* already mentioned [18] together with Livingston's triplet–triplet absorption experiments [24] confirm that a substantial number of excited molecules do in fact arrive in the triplet state.

Reactions involving free oxygen

A number of very general phenomena involving oxygen and excited molecules are known, and appear to put reactions of oxygen in a special class, very often involving the triplet state. Among the most important are :

(1) Quenching of fluorescence by oxygen—dissolved or in the gas phase.

(2) Photosensitised oxidation—where oxidation reactions, which either do not occur at all or occur only slowly in the absence of light, are promoted in the presence of a light-absorbing sensitiser molecule which is usually not destroyed.

(3) The photodynamic effect—a phenomenon showing many of the characteristics of sensitised photo-oxidation in which profound biological effects are produced in living organisms treated with a sensitiser (usually a dye molecule), and irradiated in the presence of oxygen.

(4) The many reversible oxygen-carrying reactions—both biological and artificial (hæmoglobin, hæmocyanin, cobalt(II) histidine, etc.). These do not obviously involve excited states, but there is evidence to suggest that excitation may well be involved in the biological cases.

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[38] Evstigneev, Gavrilova, and Krasnovski, *Doklady Akad. Nauk*, 1950, **74**, 315.

[39] Krasnovski, *Zhur. fiz. Khim.*, 1956, **30**, 968.

Quenching of Fluorescence.—The quenching of the fluorescence of many substances follows the Stern–Volmer equation

$$(F_0/F) - 1 = k[\mathrm{Q}] \quad . \quad . \quad . \quad . \quad (2)$$

where F_0 is the quantum yield of fluorescence in absence of quencher, Q, and F the yield in presence of quencher. k is the quenching constant, and [Q] the concentration of quencher.

The relation is easily derived on the basis of the following steps.

Excitation	$\mathrm{X} + h\nu \longrightarrow \mathrm{X}^*$ . .	(3)
Emission	$\mathrm{X}^* \longrightarrow \mathrm{X} + h\nu'$ . .	(4)
Solvent and internal quenching	$\mathrm{X}^* \longrightarrow \mathrm{X}$ . . .	(5)
External quenching	$\mathrm{X}^* + \mathrm{Q} \longrightarrow \mathrm{X} + \mathrm{Q}$.	(6)

The rates of reactions (4), (5), and (6), respectively, are $k_f[\mathrm{X}^*]$, $k_i[\mathrm{X}^*]$, and $k_q[\mathrm{X}^*][\mathrm{Q}]$, where the k's are for the fluorescence process, internal + solvent quenching, and quenching by the added quencher, respectively. Thus we have

$$F_0 = k_f/(k_f + k_i) \quad . \quad . \quad . \quad . \quad (7)$$

$$F = k_f[\mathrm{Q}]/(k_f + k_i + k_q)$$

$$(F_0/F) - 1 = k_q[\mathrm{Q}]/(k_f + k_i) = k[\mathrm{Q}] \quad . \quad . \quad (8)$$

This simple relation is derived on the basis of direct excitation of X (no energy transfer) and a single excited state. It must be modified to satisfy more complex conditions, but it holds quite well in the simple form in many cases.

If the Stern–Volmer relation is assumed to be valid, it is clear that absolute values of the quantum yield F_0 allow k_i to be obtained from eqn. (7), since k_f is simply the reciprocal of the true lifetime of fluorescence, and may be obtained from the integrated extinction coefficient.

Many experimental investigations lead to the conclusion that oxygen is especially efficient as a quencher of fluorescence. A recent investigation [40] suggests that very few excited molecules can withstand more than one or two collisions with oxygen without deactivation.

This high efficiency is undoubtedly bound up with the triplet nature of the oxygen ground state and may occur in one of several ways, all essentially physical :

(1) By excitation of the oxygen.

(2) By the formation of an oxygen complex, with a low vibrational frequency facilitating conversion of electronic into vibrational energy.

(3) By facilitating the singlet–triplet conversion because of its associated magnetic field. Since phosphorescent emission from the triplet state is very rarely observable at room temperature, competitive quenching processes being more probable than the *T–S* transition, the observed result is simply quenching of fluorescence. This point of view is upheld by Stevens's work [41] on the quenching of aromatic hydrocarbon fluorescence by oxygen and by nitric oxide.

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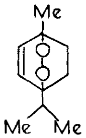
[40] Funt and Neparko, *J. Phys. Chem.*, 1956, **60,** 267.

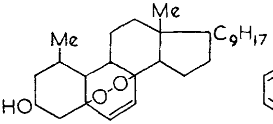
[41] Stevens, *Trans. Faraday Soc.*, 1955, **51,** 610.

The interaction of oxygen with the triplet states of excited molecules is usually of a more chemical nature, and will be dealt with in the next section.

Sensitised Photo-oxidation via *the Triplet State.*—One of the areas which has been most systematically investigated is that of the photochemical formation of trans-annular oxides. The first of these to be identified was ascaridole (I), a naturally occurring peroxide which is the principal constituent of chenopodium oil [42] and has since been prepared by a photosensitised oxidation.



(I)



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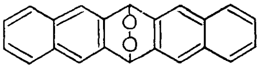

(III)

The first well-authenticated observation of formation of a trans-annular peroxide by a photosensitised reaction is that of Windaus and Brunken,[43] that ergosterol is converted into a photo-oxide in the presence of light, oxygen, and eosin, the last acting as sensitiser. The structure of the peroxide was disputed for many years, but there now seems little doubt that it is (II). Other such peroxides have since been prepared in the steroid series.[44]

The third group of trans-annular peroxides, which have been known for many years, is that of the linear polyacene aromatic hydrocarbons. This began with Clar's suggestion [45] that the readily-obtained photochemical peroxide of pentacene (III) has the structure shown. The work of Moureu, Dufraisse, and their collaborators [46] has shown that the photo-oxides of many substituted linear polyacenes (including anthracene, but not naphthalene) may be prepared, but that those of non-linear polyacenes are very difficult to obtain. This result shows a striking parallelism with the height of the lowest triplet state above ground level, that of anthracene and its higher homologues lying at much lower energy than that of non-linear polyacenes with many more rings.[47, 48]

In many of these investigations, sensitisers—the most popular of which was eosin—had been used, but the function of the sensitiser was improperly understood. However, as long ago as 1943, Terenin,[49] when discussing the then-established evidence for the existence of a long-lived triplet state produced on excitation of solutions of aromatic substances, stressed the probable importance of such a state in many of the known photochemical oxidation and polymerisation reactions. He also introduced the idea of

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[42] Wallach, *Annalen*, 1912, **392,** 59 ; Nelson, *J. Amer. Chem. Soc.*, 1913, **35,** 84.

[43] Windaus and Brunken, *Annalen*, 1928, **460,** 225.

[44] Bergman and McLean, *Chem. Rev.*, 1941, **28,** 367.

[45] Clar and John, *Ber.*, 1930, **63,** 2967.

[46] Moureu, Dufraisse, and Dean, *Compt. rend.*, 1926, **182,** 1440.

[47] Pariser, *J. Chem. Phys.*, 1956, **24,** 250.

[48] Padhye, McGlynn, and Kasha, *ibid.*, p. 589.

[49] Terenin, *Acta Physicochim. U.R.S.S.*, 1943, **18,** 210.

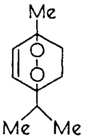
"paramagnetic quenching" of fluorescence by oxygen, involving the depopulation of the fluorescent singlet state by singlet–triplet conversion brought about in the field of the oxygen's unpaired electrons.

Approaching the subject from a very different angle, Schenck [50] postulated for photoperoxide formation a similar mechanism which may be summarised as :

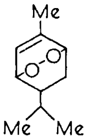
$$\begin{tikzcd}
h\nu + S \arrow[r] & S^* \arrow[r] & {}^3S^* \arrow[d, "O_2"] \\
S + AO_2 & SO_2A \arrow[l] & SO_2 \arrow[l, "A"']
\end{tikzcd}$$

where S is the sensitiser substance and A the substrate to be oxidised. The rôle of the sensitiser thus becomes that of a carrier of reactive oxygen to the substrate, allowing the oxidation to proceed by a considerably lowered barrier. By analogy with the results of zinc chlorin and porphin obtained by Calvin and his associates, we may assume that in unsensitised photochemical oxidations the substrate itself may be oxidised to the triplet state and again the reaction with oxygen can proceed with a lower energy of activation.

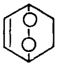
In more recent work, Schenck and his collaborators [51] have reported many new photochemical oxidations sensitised by eosin, and regard their work as having established a quite general diene synthesis with oxygen in the presence of light and eosin. Among the substances successfully prepared are ascaridole (IV), the corresponding peroxide from α-phellandrene (V), and the peroxide (VI) from *cyclo*hexadiene. In a long series of papers many other examples of trans-annular complexes are uncovered.



(IV)



(V)



(VI)

The Photodynamic Effect.—The term "photodynamic action" has been applied principally to lethal or inhibiting effects brought about on living organisms in the presence of light, oxygen, and some sensitising substance, often a dye, but not necessarily so, which absorbs the light.

It is probable that the only difference between the chemical processes involved in the photodynamic effect and in the photosensitised oxidations described in the last section is that the former occur in living systems. Consequently a mechanism involving the triplet state is again very probably involved.

The earliest observations were of hæmolysis produced by visible light

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[50] Schenck, *Naturwiss.*, 1948, **35**, 28.

[51] Schenck, Kinkel, and Mertens, *Annalen*, 1953, **584**, 125 ; Schenck, *ibid.*, p. 156 ; Schenck, Eggert, and Denk, *ibid.*, p. 177 ; Schenck and Schmidt-Thomée, *ibid.*, p. 199 ; Schenck and Ziegler, *ibid.*, p. 221.

in the presence of eosin and oxygen, and of the increased death rate of irradiated *Paramecium caudatum* in the presence of oxygen and various dyes. It has been well established that in most experiments of this kind all three components are necessary for appreciable effects to be observed. The most obvious theory seems to be that the excitation energy is handed on from the sensitiser to the oxygen, producing what has been called " active oxygen ". This could well be oxygen in its $^1\Sigma_g^+$ state, the excitation of which requires only 37·5 kcal. (7623 Å). If this is indeed the mechanism, there should be a marked fall off in photodynamic efficiency as the exciting light falls in energy below this value, even though a suitably absorbing dye is used. The only experiment to test this hypothesis is that of Gaffron,[52] who found bacteriophæophitin sensitive to 8000 Å, suggesting that the $^1\Sigma_g^+$ state of oxygen is not involved unless it is very considerably shifted in position, as is possible in a molecular complex of oxygen and the dye. It may thus be that a labile compound of oxygen and the excited dye molecule is formed, and is itself the active oxidising agent.

At all events, oxygen is consumed and its consumption does not result in production of carbon dioxide, the respiratory quotient (*i.e.* the ratio carbon dioxide produced/oxygen absorbed) falling from approximately unity to as low as 0·05. The oxygen uptake under photodynamic conditions continues even at high temperatures, and in the presence of inhibitors such as cyanide, when normal oxygen uptake in respiration falls off.

The characteristics of photodynamic reactions are that they are more or less independent of temperature, are irreversible, and their progress is proportional to total energy absorbed (intensity × time, for a given energy source) although the efficiency falls somewhat at very high light intensity. The rate is of zero order with respect to the concentration of dye-sensitiser except at very low concentrations. The implication of this last fact is that the dye molecule may be used many times to activate the reacting oxygen molecules and may thus be considered as a true catalyst. The quantum yield of most photodynamic processes (expressed as oxygen reacted/quanta absorbed) is close to unity, although the efficiency falls at low substrate concentrations. This is to be expected if some of the excited molecules are "wasted" because they are deactivated before meeting a substrate molecule.

Various intermediates have been suggested as playing a part in the process, dye peroxides, HO_2 radicals, and hydrogen peroxide being the most favoured. No such intermediates have been positively identified, however, and it is possible that the same mechanism is not always operative.

A clue to possible mechanisms is provided by the nature of the photosensitive molecule. Substances as different as aromatic hydrocarbons, methylene blue, flavines, porphyrins, etc., seem equally effective.

All the dyes showing strong photodynamic effects are fluorescent. However, this fact tells us little about their mode of action. Usually the existence of fluorescence means that the excited state persists for at least 10^{-8} sec. without radiationless deactivation to the ground state. Thus it has the opportunity for $\simeq$ 1000 collisions, and a reasonable chance of meeting an

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[52] Gaffron, *Biochem. Z.*, 1936, **287**, 130.

oxygen molecule, if excited dye + oxygen molecule is the requirement. On the other hand, dyes which are normally non-fluorescent have an efficient internal conversion system (probably intersection of the ground- and excited-state potential curves), and become deactivated in times shorter—and possibly very much shorter—than 10^{-8} sec. The chance of such molecules meeting an oxygen molecule while still excited is correspondingly reduced.

Once again, however, it seems more likely that the reactive species is the triplet state of the sensitiser molecule. The correlation of fluorescence with photodynamic activity therefore appears simply because persistence of the excited state long enough for fluorescence usually means that it also lives long enough for singlet–triplet conversion into the long-lived metastable state. Of course, fluorescence with a quantum yield of unity would indicate that no conversion into the triplet state occurs, but the investigations by Lewis and Kasha, and subsequent work carried out largely by the Kasha school, suggests that this is rarely the case. Most of the non-fluorescent and non-photodynamically active dyes must therefore be non-fluorescent because of complete and rapid internal conversion into the ground state rather than into the triplet state. A crucial experiment on this subject would be a comparison of the relative photodynamic efficiency of related substances in which the fluorescence : phosphorescence ratio was changed by heavy-atom substituents. No such work has yet been reported. The work on the enhancement of singlet–triplet transitions in the presence of oxygen, described in the next section, raises the possibility that this may in fact be the principal reason for the " oxygen effect ". This implies that the long-lived triplet radical is the damaging species, and that the oxygen's rôle is the purely physical one of increasing the spin-orbit coupling.

Enhancement of S–T *Absorption by Oxygen.*—Some years ago Evans [53] observed that many organic solvents showed absorption at larger wavelengths than usual in the presence of dissolved oxygen, and that, when the oxygen was swept out by a current of nitrogen, this absorption disappeared. The phenomenon was at first attributed to a " charge transfer " absorption band, similar to that found for instance in molecular complexes between the halogens and benzene. A more careful investigation of the new absorption in the oxygen–benzene system [54] showed that the singlet–triplet transition located at 3300 Å, which had already been observed both in emission and weakly in absorption, showed a much enhanced adsorption band in the presence of oxygen, that vanished completely when the oxygen was swept away. Evans concluded that this was due to the spin-orbit coupling effect, and has since investigated the effect of oxygen at pressures up to nearly 100 atmospheres on the singlet–triplet absorption of many molecules (benzene, and many polycylic aromatic hydrocarbons and their halogenated derivatives, acridine, styrene, *trans*-stilbene, and diphenylacteylene).[55] The increase in intensity is very marked and appears to correspond to about a fifty-fold increase in S–T extinction coefficient at 75 atmospheres. Oxygen thus appears to be more efficient than is, for instance, the iodine atom in

[53] Evans, *J.*, 1953, 345.

[54] *Idem, Nature*, 1956, **173**, 536.

[55] *Idem, J.*, 1957, 1351.

ethyl iodide when the latter is used as a solvent, or than the paramagnetic gadolinium ion. Whether or not this is because of the formation of well-defined molecular complexes with the oxygen, has not been clearly established, although quantitative measurements of the enhancement of absorption intensity in *cyclo*hexane by oxygen have been interpreted in this way.[56]

Long-lived phosphorescence of proteins

There are numerous reports of fluorescence, and a few of phosphorescence, in protein systems. In general, however, the difficulties of assuring purity in such systems make the positive identification of any of the long-lived emissions with triplet levels open to doubt. It is perhaps significant [57] that most of the reports on long-lived emission at room temperature come from dense, near-crystalline materials such as tendon and cartilage. This suggests that the emission may often be from a " trap " different in nature from the bulk of the material and that a " solid-state " phenomenon involving exciton transfer occurs. If the phenomenon were a true molecular one, we might expect less emission from such compact materials than from less dense materials because of increased concentration quenching. In fact, less compacted materials (globulins, etc.) show no phosphorescence at room temperature.

At low temperature (77° K) phosphorescence is much more general in protein systems. Bovine-serum albumin, egg albumin, γ_2-globulin, gelatin, keratin, zein, fibrinogen, and silk fibroin all show a characteristic blue phosphorescence. Debye and Edwards [58] found that of eighteen amino-acids investigated only those containing an aromatic ring (tyrosine, tryptophan, and phenylalanine) showed appreciable phosphorescence, and attributed this to a triplet–singlet transition.

These authors observe also that most proteins show at least two separate exponential-decay processes and a band shape which is dependent on pH. The tryptophan spectrum (lifetime 3 sec.) is pH independent, but that of tyrosine has a lifetime of 3 sec. in acid medium, which falls to about 0·9 sec. in alkali. The complexity of the spectrum of protein may thus be due to a superposition of these two, with perhaps some contribution from phenylalanine, which has a much weaker phosphorescence, with a lifetime less than 0·1 sec.

In the materials studied so far, the shape of the emission band in alkali is almost independent of the nature of the protein. If, indeed, both tyrosine and tryptophan contribute towards it, this suggests that the ratio of these components must be approximately constant in the proteins studied.

The observation of phosphorescence, of course, means that excitation energy is being wasted, whereas our main interest is in cases where it is used to promote a chemical reaction. In this connection it was observed by Harris [59] that most proteins take up oxygen more readily when irradiated

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[56] Munch and Scott, *Nature*, 1956, **177**, 587.

[57] Giese and Leighton, *Science*, 1937, **85**, 428.

[58] Debye and Edwards, *ibid.*, 1952, **116**, 143.

[59] Harris, *Biochem. J.*, 1926, **20**, 288.

than in the unexcited state. In particular, just these two amino-acids, tyrosine and tryptophan, absorb oxygen rapidly under these conditions. Once again it seems very reasonable to suppose that the excited triplet state which persists long enough for 10^8 or more collisions may react with oxygen, which itself has a triplet ground state, very readily.

Recently, Szent-Györgyi[60] has reported that enhanced, or entirely new long-lived emissions are observed when various dye molecules are irradiated in cold frozen aqueous systems, *i.e.* in the solid crystalline state rather than in the usual glassy media, and has suggested that this phenomenon may also occur in the " pseudocrystalline " ordered water of biological systems. It may be that some new mechanism of *S*–*T* conversion involving the crystal field is involved, or simply that competitive quenching mechanisms are better eliminated in the more rigid crystalline medium. It should be possible to distinguish between these two effects since the first should lower, and the second raise the observed lifetime. No measurements have yet been reported. It must be mentioned, however, that in frozen crystalline systems spurious effects—such as crystallisation of particles of the dye itself, which may show emission different from the solution—are possible, and were not considered by Szent-Györgyi.

It may be said in conclusion that while the triplet state levels and some triplet state reactions have been characterised for relatively small molecules of widely differing constitution, little quantitative work has been done on the phosphorescence of large molecules. The undoubtedly important rôle played by the triplet state in many biological reactions remains to be elucidated.

FIGS. 2, 3, 5, 6, and 7 are reproduced, with permission, from the Reviewer's book, " Excited States in Chemistry and Biology ", Butterworths, London, 1956.

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[60] Szent-Györgyi, *Science*, 1956, **124**, 873.