RECENT PROGRESS IN PHOTOBIOLOGY

THE PROCEEDINGS OF AN
INTERNATIONAL CONGRESS HELD AT
OXFORD, JULY 1964
UNDER THE AUSPICES OF THE
COMITÉ INTERNATIONALE DE
PHOTOBIOLOGIE

EDITED BY

E. J. BOWEN

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O quanta qualia sunt illa concilia
Adapted from Abelard, 1100 A.D.

EARLIER INTERNATIONAL PHOTOBIOLOGY CONGRESS VOLUMES

Proceedings of 1st International Photobiology Congress
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Proceedings of 2nd International Photobiology Congress
MINERVA MEDICAL MONOGRAPH 1957

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PREFACE

The 4th International Photobiology Congress, under the auspices of the Comité International de Photobiologie, and organized by a committee set up by the British Photobiology Group, was held in Oxford, 26–30 July 1964. Over 500 members, from 29 countries, attended and 234 papers were presented. The proceedings comprised Rapporteur Sessions with Introductory Lectures, Sectional Meetings and Symposia. The papers presented will be submitted by their authors to appropriate scientific journals; this volume is a record of the lectures, reports and discussions representing the co-operative activities of the Congress. The subject, a wide one, as will be appreciated from a study of the contents of this volume, is at present in a stage of rapid development, particularly in the interpretation of observed facts in terms of biological structures, chemical changes and molecular energy levels, and the aim of the Congress was to assist in the unification of the many branches of photobiological research. Every effort has been made to reproduce as accurately as possible what the various contributors intended to convey, and the Editor must be held responsible for errors and inaccuracies. Some degree of uniformity of nomenclature has been attempted; wavelengths are given in units of nm ($=10^{-9}$ meter $=1$ mμ $=10$ A), and various terms describing the spectral region below 400 nm have been replaced by the symbol u.v.

The arrangements for the Congress were in the hands of the Secretaries, Dr Daphne Vince and Dr H.J.A. Dartnall, the Treasurer, Dr S.Y.Thompson, and Mr C.F. Seath, assisted by Mrs E.M. Lewis. The help and advice given by Dr Edna Roe, Secretary General of the C.I.P., must also be acknowledged.

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SECTION I

BASIC PHOTOCHEMISTRY IN RELATION TO PHOTOBIOLOGY

INTRODUCTORY LECTURE

A. TERENIN
Leningrad University, U.S.S.R.

The main types of photochemical processes, observed in liquid solutions, are subjected in photobiology to specific changes and complications arising from the heterogeneous character of the medium and the fixation of the reactants on the biopolymer. Nevertheless, valuable information can be obtained from more simple model systems, for example in frozen rigid solutions and even in the gas phase, where we can unambiguously identify the primary act of excitation and bond fission in a large organic molecule. This presentation is restricted to some recent topics in which we are involved and which have direct implications for photobiology.

1. DISRUPTION AND IONIZATION OF MOLECULES BY VACUUM U.V. RADIATION

The range of wavelengths from 180 to 90 nm, equivalent to photon energies from 155 to 310 kcal/mole, represents a borderland between X-rays and the ordinary u.v. Most biological entities and structures in this range have absorption coefficients much higher than those in the usual spectral ranges. Photochemical efficiencies in the vacuum u.v. are also high, quantum yields of 1 being reported, for example for enzyme photoinactivation (SETLOW *et al*, 1960).

The photolysis of aromatic amines, the amino acids and the nitrogeneous bases by vacuum u.v. radiation, which we are studying in the gas phase by means of mass-spectrometry, shows the existence of three main photoprocesses (TERENIN and VILESSOV, 1964):

(1) electron abstraction without a disruption of the parent molecule at a threshold $h\nu_1$:

$$R_1 - R_2 \xrightarrow{h\nu_1} R_1 - R_2^{+} + e \qquad (1)$$

where R_1 and R_2 indicate constituent groups, joined by a covalent linkage;

(2) photolysis into an ionized fragment and a neutral hydrogen atom, or radical, which begins at a higher photon threshold $h\nu_2$:

$$R_1-R_2 \xrightarrow{h\nu_2} R_1^{+}\!\cdot + \cdot R_2(\cdot H) + e \qquad (2)$$

(3) disruption of cyclics at still higher energies $h\nu_3$:

$$R_1-R_2 \xrightarrow{h\nu_3} r_1^{+}\!\cdot + \cdot r_2 + e \qquad (3)$$

The electron photoemission from the molecules, process (1), helps to obtain values of the ionization energies, which parallels the electron donating properties of the molecules concerned, and this information is of primary importance for understanding intermolecular electron transfer reactions.

In process (2) the molecular positive ion, which has acquired a definite excess of energy above the work done to remove an electron, breaks into a positively charged fragment and a neutral particle in a time less than 10^{-7} sec.

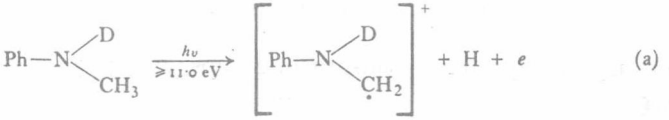
As an example, glycine at a photon energy of 9·6 eV is disrupted into the following fragments, the first one being observed in the mass spectrum:

$$H_2N{-}CH_2{-}COOH \xrightarrow[\geqslant 9{\cdot}6\,eV]{h\nu} H_2N{-}CH_2^{+}\!\cdot + \cdot COOH + e$$

The most remarkable fact in this ionic dissociation process is that in several cases the energy excess imparted to the molecule over the work of electron abstraction is only a fraction of the ruptured bond strength, as known for the unionized neutral molecule. Thus the disruption energy for glycine above is only 0·6 eV as compared with 3·7 eV, the normal C—C bond strength. This means a significant redistribution of the electron density in the ion formed, leading to an abnormal reduction of bond strength at definite linkages.

A similar loosening of the C—C bond has been observed for α- and β-alanine, and β-phenyl alanine. In these cases one can presume that the ionized fragment $H_2N{-}CH_2^{+}$ assumes the valency configuration $H_2N^{+}{=}CH_2$, with a corresponding energy gain.

A similar dissociative photoionization has been found by us for *N*-methylaniline, partly deuterated in order to identify the site of H atom elimination.



(a)

The C—H bond in the methyl end group is broken, but not the Ph—N or N—CH_3 bonds. Cases of bond splitting in a side chain are known for benzene derivatives, photolysed in frozen rigid solutions by light in the ordinary u.v. range (PORTER *et al*, 1955–63). In case (a) the excitation energy imparted to the ionized molecule amounts to 3·66 eV, which is sufficient to split up a normal C—H bond. However, benzylamine is photolyzed with the elimination of an H atom from the connecting methylene group:

$$\mathrm{Ph{-}CH_2{-}ND_2} \xrightarrow[\geqslant 9{\cdot}4\,\mathrm{eV}]{h\nu} \mathrm{Ph{-}\dot{C}H{-}ND_2^{+}} + \mathrm{H} + e$$

The energy excess $\Delta E = h\nu - I_p$ imparted to the molecular ion at the threshold is only 0·8 eV, which is certainly well below the usual C—H bond strength (3·4 eV). Similar effects have also been found for the hydrazines in our laboratory (AKOPIAN and VILESSOV, 1963). Evidently, as in the former example, an electronic rearrangement in the fragments has taken place with a gain in energy compensating the deficiency. A valency redistribution of the ionic fragment into $\mathrm{Ph{-}CH_2{=}\overset{+}{N}D_2}$ may be suggested.

At the higher energies (> 11 eV ≈ 250 kcal/mole) drastic cleavage processes occur with opening of the heterocyclic rings of pyridine, γ-picoline, uracil, etc. In the aromatic amines the part primarily affected by the photon is the phenyl 'chromophore', but in the very short u.v. spectral range here considered the NH_2 and OH groups can be the directly absorbing ones.

II. SELF-IONIZATION OF THE PHOTOEXCITED MOLECULE

The abnormally long (up to 0·01 sec) delayed fluorescence spectrum of aromatic amines, acridine and its derivatives, carbocyanin and fluorescein dyes in frozen rigid solutions (77°K), has been satisfactorily explained as due to the detachment of an electron from the excited singlet molecule, its trapping by the medium, and to the slow recombination process with the positive ion, an excited molecule being reformed (LEWIS *et al*, 1942; DEBYE *et al*, 1952; LIM *et al*, 1962–63; KERN *et al*, 1962; SHABLIA *et al*, 1964; KALANTAR *et al*, 1962; KROG *et al*, 1963). The same process has been observed in frozen alkaline serum albumin, in amino acids, pyrimidine, purine bases, nucleic acids, etc. and is of general occurrence (DUMARTIN *et al*, 1957; KATIBNIKOV *et al*, 1962; MEKSHENKOV *et al*, 1961; KONEV

et al, 1961; BURSHTEIN, 1961; IMAHORI *et al*, 1959; ROSENHECK *et al*, 1961).

The yield of such delayed fluorescence is of the order of 1 per cent of the normal fluorescence and increases at wavelengths shorter than the maximum of the first absorption band of the molecule. This suggests that some small activation energy must be additionally spent from the absorbed photon to achieve this remarkable electron detachment with an energy value of only 3–4 eV, i.e. about half the ionization potential of the free molecule (7 eV). It should be noted that it has been recently found that although the absorption of one photon is sufficient

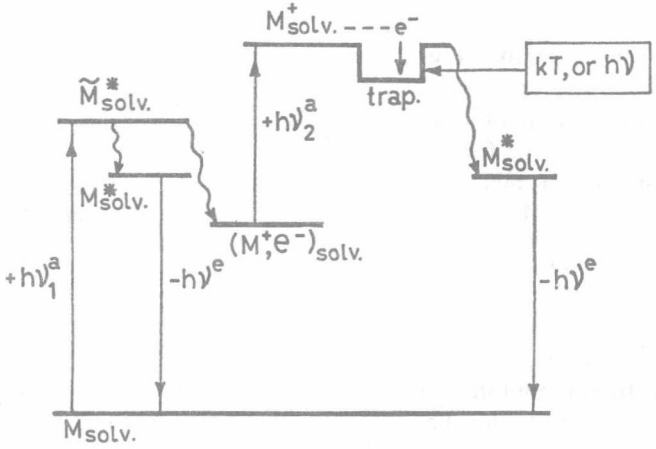


FIG. 1. Diagram of the consecutive steps involved in the delayed fluorescence caused by electron recombination.

to detach the electron from the parent molecule the absorption of a *second* photon is required in order to remove it from the proximity of the parent molecule and destroy this primary charge transfer complex (KALANTAR and ALBRECHT *et al*, 1962).

The two-step process of ionization can be thus represented by the sequence of events shown in Fig. 1.

III. BI-PHOTONIC PHOTOCHEMICAL PROCESSES

There exist two-step photochemical processes of another kind.

The photochemical activity of organic molecules in their triplet state, in particular that of chlorophyll, hematoporphyrin, riboflavin etc., has been widely reviewed and does not require extended comment

here. Recently several photochemical processes have been found which have demonstrated that a conversion of the photoexcited molecule to the triplet state is prerequisite, but that for the reaction a second photon must be absorbed by the triplet molecule. The rate of such photochemical reactions then becomes proportional to the *square* of the light intensity, instead of to the usual first power relationship to which photochemists have traditionally accustomed themselves.

Three types of processes of such *intensity non-linear* photochemistry have recently been found. They have been studied in frozen solutions by e.p.r. and phosphorescence methods.

(1) The photodissociation of triphenylmethane, its chloride, carbinol, etc. with the production of the triphenylmethyl radical $Ph_3C\cdot$ (KOZLOV *et al*, 1963).

$$Ph_3C{-}X \xrightarrow{h\nu_1} Ph_3C{-}X^{*\rightarrow T} \xrightarrow{h\nu_2} Ph_3C\cdot + \cdot X (X{=}H, Cl, OH)$$

In this photolysis the energy requirement for the splitting of the feeble (ca. 50 kcal/mole) C—R (R=H, Cl, OH) bond should be already met by one photon excitation, as observed in the initial stage, when the concentration of triplet molecules is low. The bi-photonic mechanism dominates at a later stage.

(2) The alkylbenzenes (toluene, etc.), di- and tri-phenyl methane, etc. act as photosensitizers evolving hydrogen from saturated hydrocarbons (e.g. 3-methylpentane) in which they are dissolved. This bi-photonic reaction proceeds for toluene through the following sequence of steps (VINOGRADOVA *et al*, 1964):

$$\underset{\text{deutero toluene}}{PhCD_3} \xrightarrow{h\nu_1} PhCD_3{}^{*\rightarrow T} \xrightarrow{h\nu_2} \underset{\text{hydrocarb. solvent}}{PhCD_3{}^{T'}, HR} \longrightarrow PhCD_3 + H\cdot + R\cdot$$

$$H\cdot + HR \longrightarrow H_2 + R\cdot\,(94\%)$$

$$H\cdot + PhCD_3 \longrightarrow HD + Ph\dot{C}D_2\,(6\%)$$

It has been kinetically proved that triplet molecules (^{T}) are the active ones, and that a second photon is required to reach a higher triplet ($^{T'}$) level in order to break a H—C bond in the hydrocarbon solvent by energy transfer. Ninety-four per cent of hydrogen is evolved as H_2; the remaining 6 per cent in the HD form is ascribed to the secondary process shown. The radical $R\cdot$ is detected by its e.p.r. spectrum.

(3) H atoms are likewise abstracted from the alcohols by the photoexcited aromatic amines (diphenylamine, triphenylamine, *N*-methyldiphenylamine) and the *N*-heterocycles (carbazole, indole, tryptophane, porphyrines and flavines, etc.) in a bi-photonic process (SMALLER, 1963; HOLMOGOROV *et al*, 1963; BAGDASSARIAN *et al*, 1963; BAJIN *et al*, 1964; PISSKUNOV *et al*, 1964; GRIBOVA *et al*, 1963; PTAK *et al*, 1963). We presented the following interpretation for triphenylamine (HOLMOGOROV *et al*, 1963):

$$Ph_3N \xrightarrow{h\nu_1} Ph_3N^{*}\rightarrow T \xrightarrow{h\nu_2} \underset{7\ eV}{Ph_3N^{(+)}e,CH_3OH} \longrightarrow Ph_3N + H\cdot + \cdot CH_2OH$$

The mechanism consists either in a transient H transfer to a high triplet state of the amine with the formation of a short-lived reduced form, or in an energy transfer to the C—H bond of the alcohol, similarly to the former photosensitized reaction (2). Experiments with deuterated alcohols seem to show that quite unusually it is the H atom from the hydroxyl group which is abstracted, not the one from C—H in the α-position (PISSKUNOV *et al*, 1964). This latter H atom is presumably split from a second alcohol molecule by the R—O· radical, primarily formed.

By selective deactivation with added triplet energy acceptors it has been experimentally proved that the triplet molecule is involved (HOLMOGOROV *et al*, 1963). The second photon must be a large one and, in fact, exceeds the height of the second triplet level. A very high energy level must thus be reached and the sensitizer molecule might even be ionized by this process of *two-fold* excitation. In fact, the ionization potential level of 7 eV (160 kcal/mole) becomes accessible by the successive absorption of two photons in the near u.v. range (ca. 350 nm, equivalent to 80 kcal/mole).

Moreover, we observed the doublet e.p.r. signal of the H atom (HOLMOGOROV *et al*, 1963) provided the atoms are stabilized by adsorption on silica gel, which was introduced into the illuminated solution (Fig. 2). The photogeneration of active H atoms in this reaction is also indicated by the appearance, on the addition of benzene to the solution, of the s.f.s. e.p.r. band of the radical $\dot{C}_6H_7$ together with that of $\cdot CH_2OH$ (Fig. 3).

There is no necessity to use extremely high light fluxes to observe such *photon summation*, since, as is well known, in frozen rigid solution

the lifetime of the triplet state reaches several seconds, and the stationary concentration of triplet molecules attains to a high proportion of all the molecules present. A high population of the upper state may be easily accomplished with ordinary light sources.

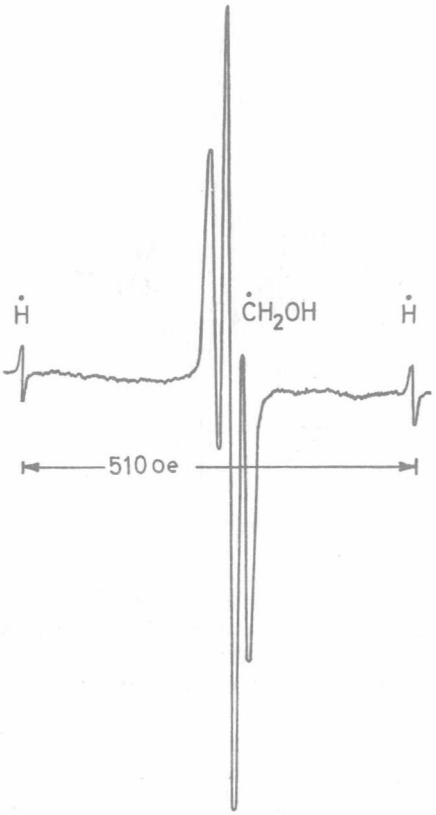


FIG. 2. Doublet e.p.r. signal of H atoms, abstracted from CH_3OH by photoexcited triphenylamine molecules, and stabilized by adsorption on silica gel. The superfine structured e.p.r. spectrum of the radical $\dot{C}H_2OH$ is also shown (77° K).

The population of the triplet state can also be efficiently achieved by a direct triplet–triplet energy transfer from a suitable photosensitizer absorbing photons of lower energy than those required by the parent molecule, for example benzophenone for indole (SMALLER, 1963).

The implications for photobiology of these recent findings is obvious. Under conditions of a rigid framework of biopolymers and anaerobic conditions, the lifetime of the triplet state of embedded chromophoric groups is definitely increased even at room temperature.

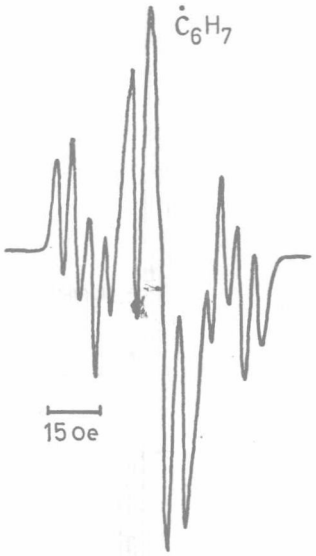


FIG. 3. Superfine structured e.p.r. spectrum of the radical $\dot{C}_6H_7$, formed by addition to benzene of an H atom, abstracted from the methanol solvent by photoexcited triphenylamine. The e.p.r. spectrum of the $\cdot CH_2OH$ radical is also present in the centre (77° K).

This is in particular shown by the slower decay of the 'exponential' triplet phosphorescence, due to inclusions and imperfections, in proteins or nucleic acids. Therefore favourable conditions for biphotonic bond cleavage, dehydrogenation and local ionization are to be expected.

IV. MODES OF EXCITATION ENERGY PROPAGATION IN BIOSYSTEMS

1. The inductive resonance type of singlet excitation energy transfer over large distances, of the order of 5 nm, well known from the luminescence of dissolved molecules (FÖRSTER, 1959), requires in a macromolecular array the presence of electronically self-consistent chromophoric groups possessing similar levels and large transition

dipoles. This is the case for the well known excitation transfer between the aromatic amino acids in the proteins, from these acids, or from conjugated dyes in the globins to hemes on their surface, etc. (STRYER, 1960).

An important special case is the excitation transfer between two independent 'chromophoric' groups of the *same* molecule. Evidence for such has been obtained from luminescence experiments in which these independently absorbing parts are connected by single bond links, excluding any conjugation between their electronic systems.

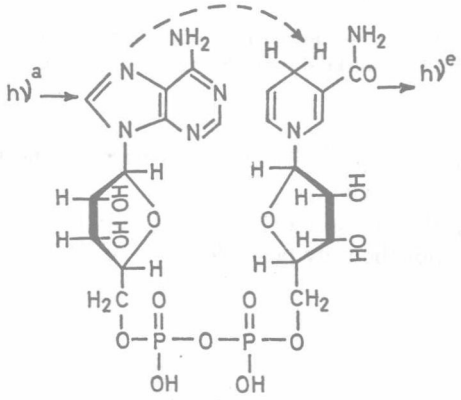


FIG. 4. Intramolecular excitation energy transfer in DPNH from the adenine group to the nicotineamide one (WEBER, 1957). (In the various Figures $h\nu^a$ and $h\nu^e$ with arrows pointing to the groups concerned refer to the absorbed and emitted photons respectively. The dashed curved arrows indicate the direction of the intramolecular transfer.)

This is reflected in the absorption spectrum which represents a superposition of the practically undisturbed spectra of the components. The free rotations in the connecting chain of such linkages allows a conformation where the chromophoric groups come into close contact and an inductive resonance transfer between them becomes possible.

This has been shown for dihydro-diphosphopyridine nucleotide (DPNH) in which photon energy absorbed by adenine is transferred with high efficiency to the nicotineamide part and emitted as fluorescence of the latter (WEBER, 1957) (Fig. 4). A similar transfer is known for flavin adenine dinucleotide (FAD) between adenine and the isoalloxazine group (WEBER, 1950).