indicated increases amounting to as much as 400 percent of what would have fallen if the Ventura generators had been inactive. The other possible interpretation is that the storms of 1957 were in some way different from those in 1958 and that the target-control relationships of precipitation from these two types of storms were very different. This phenomenon of the dependence of the target-control relationship on the type of storm has been noted elsewhere (*4*).

In order to make the results of Tables 1 and 2 more graphic, Fig. 3 was constructed. It gives the scatter diagrams of precipitation from seeded and from nonseeded clouds in the subtarget Santa Barbara North-West area and in control area A. The curves represent the precipitation to have been expected in the target area in the absence of seeding by the generators indicated.

The striking feature of Fig. 3 is that the unexpectedly high proportional increases in precipitation noted above are due not to very heavy amounts of rain on the target from seeded clouds but to the fact that spectacularly low amounts fell when there was no seeding. One possibility is that in 1957 (not in 1958) the clouds passing over Santa Barbara County had a marked deficiency of natural ice-forming particles, hence there was very little natural rainfall and an unusual increase due to seeding.

Further experimentation is needed in order to answer the many interesting questions raised by the Santa Barbara project. This further experimentation should be factorially randomized and should be broader than the experimentation reported here; it should include several physical measurements (for example, of the density of nuclei) capable of supporting or contradicting the basic hypotheses underlying cloud seeding. Also, it is to be hoped that in these further experiments it will be possible to install the all-important rain gages in locations where they can be serviced without undue hardship and, at the same time, be out of range of hunters engaged in target practice and protected from other possible interference.

References and Notes

1. This work was done in the Statistical Laboratory, University of California, Berkeley, with the partial support of the National Science Foundation, grant G-8211.
2. J. Neyman, *Science* **125**, 61 (1957).
3. The first evaluation of the results of 1957 was included in the progress report of the Statistical Laboratory, presented to the Board of Directors on 4 Sept. 1957. The estimates of the increase in rain ascribable to seeding were given in Table 4 of that report. Although these estimates were based on preliminary data then available, the general picture they presented was very similar to that given here in the first three columns of Table 1; and showed increases in precipitation by factors of 2 and more. Several months later there appeared in print an article signed by Robin R. Reynolds, chairman of the board of directors of the Santa Barbara project ["Final Report of the Advisory Committee on Weather Control" (1957), vol. 2, p. 249]. In this article it is stated that the data of 1957 indicate an increase in the target precipitation due to seeding of about 23 percent. Also, the estimate of a 23-percent increase appears in a paper-bound mimeographed booklet issued by the North American Weather Consultants, dated December 1957. Both publications describe the cooperative character of the Santa Barbara project, with the Statistical Laboratory as one of the participants, but fail to indicate the source of the estimate of 23 percent. In fact, the relevant sentences collected from page 4 of the booklet read as follows: ". . . the statistical design and analysis is being conducted by the Statistical Laboratory of the University of California at Berkeley. . . . The data for the first year have been analyzed. . . . The average increase for the first season was 23 percent. . . ." We wish to make it clear that this estimate was reached and published without our knowledge and that it bears no relation to Table 1 of this article or to the preliminary evaluation reported to the board of directors of the Santa Barbara project on 4 Sept. 1957. The two publications in which the estimate of 23 percent is given came to our attention in the spring of 1959, at which time we registered our regret. At the time of this writing (November 1959) we were informed by R. D. Elliott that, after our protest in March, the NAWC circularized the recipients of the report of 1957 requesting that the estimate of 23 percent be removed.
4. T. A. Jeeves, J. Neyman, E. L. Scott, *Bull. Calif. State Water Resources Board Publ. No. 16* (1954), p. D-1.

CURRENT PROBLEMS IN RESEARCH

Quantum Phenomena in Biology

Natural, ultraviolet, and high-energy radiation processes are compared.

C. Reid

This article is confined to the discussion of phenomena in which the absorption or emission of energy is clearly demonstrable as initiating or terminating a sequence of biochemical events. Doubtless many of the "dark" processes occurring in biological systems without such initiation or termination will one day be examined in terms of the "quantum phenomena" involved, but as yet few data are available about the participation of excited states in such reactions.

Living things have evolved in a world where light is the only widely available source of energy at a high enough potential to excite molecular electrons into new energy levels. Thanks to various absorbing layers in the upper atmosphere, most of the small fraction of solar radiation of high enough energy to cause gross rupture of chemical bonds is conveniently filtered out, leaving appreciable intensity only at wavelengths greater than 300 millimicrons. Since excited molecules often undergo reactions which are energetically unfeasible in the ground state, light is a major factor in the struggle of living organisms to decrease their entropy at the expense of the environment. It is not surprising, therefore, that the great majority of organisms—with the possible exception of a few parasitic forms—make use of light energy in one way or another.

Such processes we will call normal quantum phenomena. They may be characterized by the following features.

1) Absorption of light energy is confined to special molecules, located in special regions of the organism. This is possible because none of the major constituents of the living cell—water, proteins, nucleic acids, and their building blocks—have significant absorption at wavelengths greater than 300 millimicrons.

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2) The principal absorbers are invariably molecules with extended π electron systems, which are distinguished from molecules with only σ bonds by the fact that after excitation they remain in the excited state for appreciable periods of time before the energy is degraded to heat. These periods are often long enough for the energy to be handed on to some other species (this takes only about 10^{-11} second) or to be re-emitted as radiation (about 10^{-8} second or even longer).

3) As far as we can judge from the processes so far investigated, energy absorbed by a given kind of pigment molecule is utilized or disposed of in a unique way.

In these days of high-energy radiation, however, "normal" quantum phenomena are not the only ones to which organisms are subjected. Abnormal irradiation may be caused by (*i*) ultraviolet light of wavelength 300 to 150 millimicrons. Here absorption may still be selective (although far less so than in the visible region), since molecules differ widely in their ultraviolet absorption spectra. The absorbed energy may be so great, however, that many chemical bonds are broken, resulting in the production of ions, radicals, and electrons.

As the ultraviolet energy increases we finally reach a wavelength at which water absorbs. Radiation of higher energy than this we classify as (ii) high-energy photons (ultraviolet, x, and γ rays). Now absorption extends indiscriminately through the organism, independently of the nature of the absorbing molecules, the radiation penetrating deeper as its energy is increased.

Or, abnormal irradiation may be caused by (iii) particulate bombardment (electrons or heavy particles). The effects of electrons are often similar to those of high-energy photons, but heavy, charged particles, because of the high density of ions and radicals produced along their tracks, may have qualitatively different effects.

The difference between normal and abnormal phenomena is schematically depicted in Fig. 1.

It is clear that we must expect extremely complex effects from high-energy irradiation. Not only does the initial indiscriminate absorption process result in a host of different very reactive species, but this diversity is multiplied by the fact that secondary electrons of energies ranging from hundreds of thousands of electron volts down to thermal energies are ejected from the disrupted molecules and themselves proceed to do more damage. In view of the infinite possibilities it is surprising that any of the effects of high-energy bombardment are simple enough to be unraveled.

Normal Effects

Before dwelling further on this very complex process, I will consider the present status of the investigation of some normal quantum phenomena. Many excellent reviews (*1*) of experimental work are available, and the object of this article is to suggest what are perhaps significant trends in recent investigations.

Photosynthesis. Photosynthesis deserves first place in any account of quantum phenomena in biology, since it is no understatement to say that, without photosynthesis, life on this planet, if it could have evolved beyond very simple forms, would have been profoundly modified. Here we are not concerned with the details of the complex chain of "dark" biochemical events, many of which have been unraveled so successfully by Calvin (*2*) and others, but with the immediate results of light absorption. In spite of the intensive work of 50 years there remain many unanswered questions. If all the energy for the reduction of carbon dioxide to formaldehyde (116 kcal) is supplied by light, at least 4 quanta of light at 680 millimicrons (the longest wavelength photosynthetically active) are required. The experimentally observed efficiency is one CO_2 molecule reduced per 6 to 8 quanta absorbed, a finding which suggests that the over-all efficiency is high.

There seems little doubt, to judge from the very suggestive electron micrographs of chlorophyll grana (*3*) and the chemical evidence, that the role of chlorophyll is to act as photosensitizer in a kind of photobattery made up of alternate lamellae of lipid and lipoprotein with the chlorophyll molecules at the interface. Terenin and Putzeiko (*4*) have shown that various chlorophylls and phthalocyanins can act as photosensitizers to conductivity when evaporated as monolayers on metallic oxide surfaces. While these systems are very different from the chloroplast, the work does supply an attractive model. Terenin's oxides behaved as *p*-type conductors. There is no evidence as to whether *p*- or *n*-type conduction predominates in the chloroplast.

At all events the net result is presumably that at one electrode electrons are available, probably converting triphosphopyridine nucleotide (TPN) to the reduced form (TPNH),

$$\mathrm{TPN} + \mathrm{H_2O} + e^- \rightarrow \mathrm{TPNH} + \mathrm{OH^-},$$

while at the other electrode oxygen is liberated as the ultimate result of the reaction of the positive charge carriers

$$\mathrm{H_2O} + R^+ \rightarrow R + \mathrm{H^+} + {}^{\bullet}\mathrm{OH}$$
$$2{}^{\bullet}\mathrm{OH} \rightarrow \mathrm{H_2O} + [\mathrm{O}]$$

The subsequent cycle (*2*) involving both TPN and adenosine triphosphate (ATP) suggests that the net efficiency of the photosynthetic process is almost 85 percent for a 12-step multicycle process, a remarkably high value when compared with the quantum yields of most simple, in vitro, photochemical processes. The exact mechanism by which a photobattery of this kind might operate is still not clear. Presumably photoionization of the chlorophyll molecules (which may be from the triplet state) is a unidirectional process because charge separation through the lipid layer requires more work than does electron migration through the protein.

However there is no real evidence to rule out a "wet cell" mechanism involving ionic conduction, and there are still proponents of an energy, rather than an electron transport, mechanism. I will take up the conduction band versus intermolecular energy transport controversy below, in another connection.

The visual process. After photosynthesis, the biological quantum phenomenon which has received most attention is probably that of vision. It is worth our while to review the salient features of this complex process, if only to show that without detailed knowledge of the biochemistry involved we are likely to oversimplify our picture of the physical phenomenon. The work of Granit and others has shown that in ordinary black-and-white vision the act of illumination triggers, by discharge of the rod cell membrane, what is in every respect a normal nerve impulse. The triggering mechanism is, then, what is unique to the visual process, and particularly in view of the suggestive similarity between the lamellar structure of the rod cell contents and the chlorophyll grana (*5*), the idea that we are again dealing with a conduction band or excitation phenomenon is a very attractive one. While this may indeed be the case, the biochemistry and stereochemistry, unraveled largely by the work of Wald *et al.* (*6*), is very complex.

It was established at a fairly early stage that the illumination of rod cells resulted in the dissociation of the conjugated protein rhodopsin into the protein opsin and retinaldehyde (vitamin A aldehyde). It was natural to suppose that the triggering mechanism resulted in some way from this process. It soon became apparent that this was not a simple reversible dissociation, however. The retinene-forming rhodopsin is the 11-*cis* stereoisomer, whereas that resulting from photodissociation is the all-*trans* variety. Furthermore, two intermediates, lumi-rhodopsin (stable below −45°C) and meta-rhodopsin, were found to precede the dissociation. Finally, kinetic studies of the dissociation reaction have shown that it is too slow to account for the rather rapid triggering action involved in vision.

Attention has therefore focused on the rapidly formed intermediates, lumi- and meta-rhodopsins. Contrary to earlier suppositions, recent work by Hubbard and Kropf (*7*) shows that in both these forms the retinene has already been isomerized to the all-*trans* form, It seems, therefore, that the triggering process is connected in some way with the isomerization. Of course it is possible that this step itself only occurs after electron loss, and that the triggering process could thus still be one of simple sensitized photoconduction. No conclusive work on the photoconductivity of rhodopsin preparations or of intact rod cells has yet been carried out to establish this point. Meanwhile,

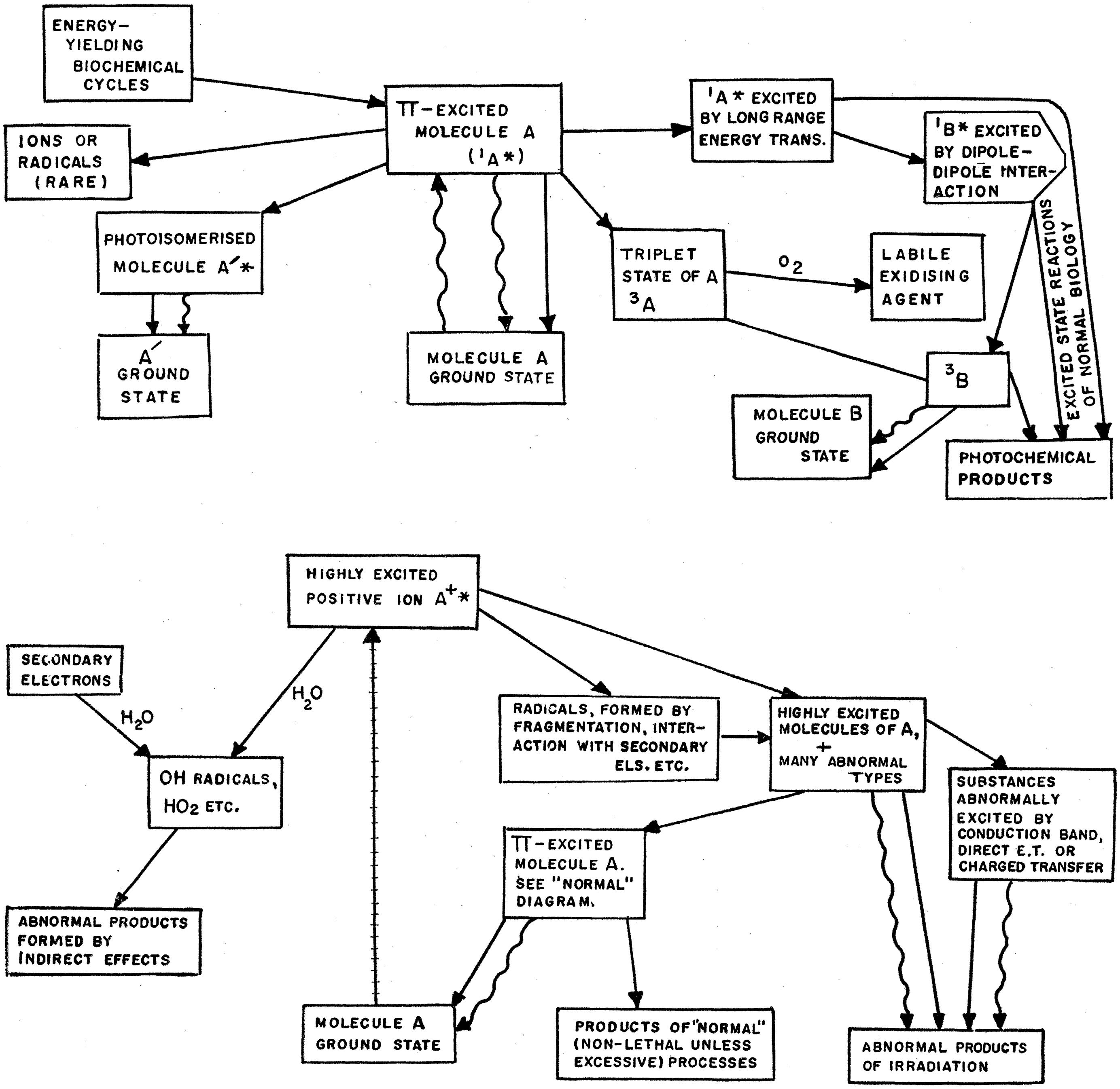


Fig. 1. "Normal" (top) and "abnormal" (bottom) excited state phenomena. The diagrams illustrate the changes in radiation-induced processes on passage from (top) visible or near-ultraviolet radiation to (bottom) high-energy ionizing radiation or particulate bombardment. Straight arrows, nonradiative process; wavy arrows, low-energy radiation; arrow with crossbars, high-energy radiation.

it is interesting to speculate that an intermolecular energy transfer process (discussed below) might be involved, resulting in the rapid passage of an "isomerization wave" from the molecule initially excited to the cell membrane.

The elaboration of a trichromatic color-vision process in the cone cells presents no insurmountable problems once the monochromatic mechanism is granted, and the study is one of cell physiology rather than of quantum phenomena per se. It is interesting to observe, however, that none of the three mammalian cone-cell pigments (these have not yet been isolated) is rhodopsin, to judge from the cone-cell spectral sensitivity curves.

Phototaxis, phototropism, and photoperiodism. None of these fascinating subjects has been sufficiently studied at the molecular level to make speculation as to physical mechanism very profitable. In a series of ingenious experiments involving very beautiful techniques, Halldal (*8*) has examined the action spectra of a number of algae and concludes that they indicate that the active substance is not one of the major cell pigments (*9*). It has often been suggested that carotenoids are involved in these phenomena. It is of some interest that carotenoids are also usually associated with the chloroplast, so it may be that they are more universally associated with photoprocesses than are the porphyrins.

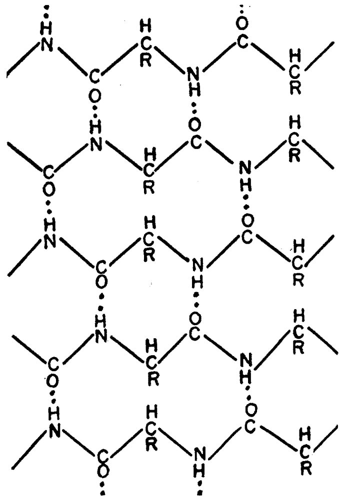
These three photoprocesses, unlike photosynthesis or vision, have to provide neither a substantial energy source nor a response faster than could usually be accounted for by a chemical phenomenon. It is possible, therefore, that they are initiated by a simple photochemical conversion in the illuminated region, all subsequent processes resulting from orthodox "dark" chemical reactions.

Energy transfer mechanisms. Before I deal with the question of abnormal quantum phenomena I shall say a few words on a subject which is relevant to the study of both normal and abnormal phenomena and which is still in a somewhat controversial state. This is the question of "energy migration"—a term which is used to indicate the transport of energy from the region of absorption of radiation to a region somewhat remote (>10 A) from this point before it is used or dissipated. Here, however, we are concerned not with a series of linked chemical transformations in adjacent molecules but with transport without chemical change, effected either by direct exchange of electronic excitation energy or by a "conduction band" mechanism.

The first of these processes might be between groups attached to the same monomolecule, or it might be truly intermolecular. The existence of some such transport in protein was postulated as long ago as 1932 by Warburg, to explain a peak in the 2600-angstrom region in the action spectrum for phototransformations in respiratory enzymes. More recently energy transfer from protein to prosthetic group has been confirmed by Bucher and Kaspers for myoglobin (from observation of the action spectrum for carbon monoxide evolution), by Bannister for phycocyanin luminescence, and by a number of workers for proteins with artificially attached luminescent dye molecules. A good review of the subject is available (*10*). All of these experiments indicated that energy absorbed in the aromatic ring systems of the phenylalanine, tyrosine, and tryptophan components of the protein was being transferred to the heme or other pigment group. The assumption was made (and this is still not unequivocally settled) that this is an example of intra- rather than intermolecular energy exchange. The question then arises as to the mechanism of the process.

The first of the two possibilities which have been most fully discussed is transport resulting from a solid-state phenomenon of some sort depending on the regular periodic (pseudocrystalline) nature of the protein molecule. Such a process results essentially from the fact that in a periodic structure with repeating groups R, the configuration R^*-R-R-R-X, where R^* is a group which has just absorbed a quantum of light, is degenerate with (that is, has the same energy as) the configuration R-R^*-R-R-X, and so forth. As the R groups are placed closer and closer together, so that their orbitals overlap strongly, a conduction band appears, first permitting only photoconduction in the presence of fairly high energy exciting light but getting closer and closer to the ground state as the R-R distance is decreased. If X is a group which can successfully dispose of the excitation energy, and any R may be excited, it is clear that random transfers occurring in times that are short compared with the lifetime of the excited state will result in funneling of energy toward X.

Any crystalline (periodic) material shows photoconduction if the energies involved are sufficiently high, and there is consequently no doubt that this kind of phenomenon is of great importance in the dissipation of the high energies involved in "abnormal" excitation. In proteins, the part of the structure with the lowest-lying conduction band is probably the hydrogen-bonded network (*11*):



Various estimates have put the energy of the conduction band in this system at 3 to 5 volts. There is no good evidence of significant amide group absorption above 1850 angstroms, however, and even at this wavelength absorption is not noticeably enhanced in proteins over the value calculated for the sum of the constituent amino acids. This would suggest >7 volts as a more probable value. There have been reports of a small increase in absorption around 2400 to 2500 angstroms, which might be due to a delocalized amide band, but these cannot be considered to have been substantiated. We must conclude, therefore, that the protein "backbone" is not involved in the transfer of energy absorbed at about 2600 angstroms to the prosthetic groups where it finally appears and is certainly not involved in any processes involving sunlight, which contains a negligible intensity of radiation of wavelength shorter than 3000 angstroms.

This brings us to the second possible mechanism. Energy may be transferred over considerable distances between identical or nonidentical molecules provided that they satisfy certain conditions without the necessity of a periodic structure. The principal requirement is that excitation energy in molecule A (the donor) shall reside for a reasonable period of time at energy levels exactly matching the energy levels of molecule B (the acceptor); that is, the two states ($A^* + B$) and ($A + B^*$) are degenerate, where A^* is an excited state of molecule A which does not too rapidly become degraded by competing processes. The criterion usually employed to decide whether this transfer mechanism can occur (*12*) is that the emission spectra of A shall overlap the absorption spectrum of B—that is, the integral

$$\int_0^\infty fe^A{}_\nu \cdot fa^B{}_\nu \, \frac{d_\nu}{\nu^2},$$

where $fe^A{}_\nu$ is the oscillator strength for emission of light by molecule A at frequency ν and $fa^B{}_\nu$ is the oscillator strength (a measure of absorption intensity) for molecule B absorbing at frequency ν, shall be non-zero. This allows self-transfer between molecules A^* and A in some cases.

Confirmation of the fact that such a process is operative in proteins comes from the fact that proteins without prosthetic groups which contain phenylalanine, tyrosine, and tryptophan show luminescence spectra characteristic of the latter (*13*). The absorption and emission spectra of the three are so situated that transfer from one to the other in the sequence given is to be expected. It is entirely reasonable to suppose that when a prosthetic group with energy levels overlapping those of tryptophan is present, the process continues. Indeed, provided that the intergroup distances are reasonable, this must be the case.

It seems, therefore, that for the case of the pigment-conjugated proteins—including most of those involved in the photosynthetic and respiratory processes—the "long-range" transfer process is the important one. It differs from what we loosely call the "conduction band" mechanism in that (i) the intermolecular distances are not necessarily equal for each transfer step, (ii) the species concerned need not be identical, and (iii) the rate of transfer is significantly lower than in true periodic phenomena, so that various other rapid deactivating processes may compete with it.

It is clear that the favorable energy-trapping configuration should be one in which the sequence of aromatic groups is one of steadily increasing wavelength of absorption as we go toward the prosthetic group, which has the longest absorption wavelength of all. So far as I am aware, the occurrence of such sequences has not yet been confirmed. Presumably one can take the comparatively high efficiency of transfer from protein to prosthetic group (*14*) as itself confirmation of the hypothesis that the favorable sequence occurs.

The foregoing experiments, however, do not rule out the possibility that cooperative effects in the hydrogen-bonded network may be important in some cases. It is known that the synchronous movement of an array of hydrogen-bonded protons can occur in certain crystals and is responsible for the ferroelectric effect in such substances as KH_2PO_4. Recent work (*15*) has shown that similar effects are possible in a molecular crystal, thiourea. We might well speculate that enzymes facilitating proton-transfer processes make use of such a step.

If this were the case, the effect of deuteration of the enzyme should be to slow down very markedly the rate of such enzyme-catalyzed reactions. This is because "hydrogen bonds" involving deuterium are considerably weaker than normal H-bonds and result in considerably increased O-N distances. This should raise markedly the energy of the conduction band. We have carried out a number of experiments to test this hypothesis. So far no marked changes have been observed for the six or seven enzymatic reactions investigated. These negative results suggest that here again no "conduction band" type of phenomena are involved.

The effect of the lowered conduction band will almost certainly show up if the relative radiation sensitivity of deuterated and nondeuterated enzymes is examined. Here the radiation employed is of high enough energy to make use of the conduction band. In experiments along these lines, positive results must still be accepted with caution, however, since they might still come from the significant structural changes recently reported by Calvin *et al.* (*16*), stemming from the weakened hydrogen-bonding network of deuterated proteins.

The role of the triplet state in biological phenomena. The last few years has shown many additions to the list of chemical processes found to proceed via the triplet state. Among these perhaps the most significant are sensitized photooxidations, many of which proceed via the pathway

$$^1S \rightarrow {}^1S^* \rightarrow {}^3S^* + {}^3O_2 \rightarrow SO_2$$

$$SO_2 + R \rightarrow RO_2 + S$$

Here $^1S^*$ represents an excited singlet state of the molecule; $^3S^*$, the corresponding triplet; and R, the substrate to be oxidized. Although SO_2, the labile oxygen complex carrying out the final oxidation step, might also be in an excited state, there is no conclusive evidence on this point.

Most of the sensitized photooxidations studied have been in vitro, but there can be little doubt that similar reactions play a part in certain biological photooxidation processes—and possibly even in some dark reactions, since the triplet levels of complex molecules may be only 20 to 40 kcal above the ground state. Direct absorption to the triplet state is weak, and hard to observe, because of a rule involving the conservation of spin angular momentum which applies fairly vigorously to optical transition. Accordingly, conditions affecting the conversion of singlet excited molecules to the triplet state in nonradiative processes are of interest to the biologist as well as to the chemical physicist. This conversion is best brought about under the effect of the magnetic perturbations brought about by the presence of heavy atoms, or more particularly, by paramagnetic species including various transition metal ions and the oxygen molecule. Kasha and Becker (*17*) have shown, for instance, that the luminescence of various porphyrins and porphyrin-like molecules is largely from the singlet state in the metal-free compound or in diamagnetic metal derivatives, but that the longer-lived triplet emission appears in the case of paramagnetic metal derivatives. Since it is still uncertain whether the photo-ionization of chlorophyll (if indeed photo-ionization occurs) is from the singlet or triplet state, or whether the triplet state plays any role in the reactions of the oxygen-carrying porphyrins, further investigation of this subject seems desirable.

What we can say with certainty is that molecules in the triplet state are very likely to take part in chemical reactions because of their long lifetimes (10^{-3} second and up) as compared with singlet states which (although they may be equally reactive chemically) usually return to the ground state with emission of radiation in less than 10^{-8} second. Furthermore, in the case of bombardment with high-energy radiation, when ions, radicals, and excited molecules are formed in profusion, the fields of ions and of radicals with unpaired electrons must facilitate singlet-triplet conversion, so triplet-state reactions no doubt play an important part in the gross reorganizations which take place after irradiation.

Still comparatively little is known about the behavior of triplet-state excited molecules in partly oriented aqueous systems. Triplet-state molecules would appear to be particularly well adapted to play a part in reactions at interfaces.

Abnormal Effects

It is still too early for the general picture of high-energy radiation effects to be very clear at the molecular level. There are, however, a number of phenomena associated with lethal ultraviolet irradiation which have received a good deal of attention. Among these I will discuss photodynamic action and photoreactivation.

Photodynamic action. This term has been applied to lethal or inhibiting effects occurring in living organisms when they are irradiated with ultraviolet or even visible light in the presence of oxygen plus some sensitizing substance, often an added dye but sometimes a natural pigment.

It seems very probable that the mechanism of photodynamic action is often, if not always, the same as the mechanism already mentioned in connection with reactions of the triplet state—namely, that the dye molecule absorbing the light energy goes into the triplet state and the triplet molecule reacts with oxygen to form a complex more efficient than free oxygen as an oxidizing agent. It seems that after the oxidation step the dye molecule may be used again in the cycle, since the reaction kinetics show zero-order dependence on dye concentration. The fact that the lethal effects of radiation often become apparent during cell division led many investigators to the conclusion that the "oxygen effect" was simply the result of an increased rate of cell division. The discovery that NO, or cupferron, could take the place of oxygen rules out this idea as a complete explanation and focuses attention on the fact that all three molecules are paramagnetic and consequently capable of facilitating singlet-triplet conversion. Just what substances are the most susceptible to oxidation during the photodynamic process has not been established. We might speculate that –SH bonds may be oxidized to –S–S– bridges, since there have been persistent reports of the special susceptibility of S-containing molecules to radiation damage.

Photoreactivation. One of the most remarkable phenomena connected with biological damage caused by ultraviolet light is that of photoreactivation. A very good review is available (*18*). Organisms which have been exposed to a dose of ultraviolet light that is usually lethal show a much reduced mortality rate if they are subsequently exposed to radiation in the visible or near-ultraviolet region of the spectrum. The effect appears to be specific for damaging radiation in the wavelength range 2000 to 3500 angstroms and is most effective from 2500 to 3000 angstroms. The reactivating wavelength is variable, presumably because the absorbing molecule varies from species to species. In general, the action spectrum for reactivation shows a maximum around 3800 angstroms, although occasionally the maximum is at longer wavelengths.

The early work on photoreactivation was carried out on bacteria and was most often concerned with survival or multiplication rate. More recently, effects have been extended in both directions—through phage to in vitro experiments on nucleotides and to gross effects on mammals. It seems very unlikely that the reaction involved is a common one in all cases, in spite of marked similarities. So far, attempts to characterize either the damaged material or the reactivator have been inconclusive. The most that can be said at this time is that the damaged material is probably deoxyribonucleic acid, ribonucleic acid, or nucleotide fragments, while the reactivator is probably protein, which may be associated with a cofactor of low molecular weight. The variable-action spectra for photoreactivation certainly do not suggest a single substance, and all kinds of molecules, porphyrins, pterins, flavins, pyridoxal derivatives, and so on, have been suggested. To all of these there seem to be objections.

If, as seems clear, there is a diversity of substances involved, we may still look for a mechanism common to them all. It is instructive to consider possible kinds of photodamage and the ways in which they might be repaired or prevented.

1) Gross damage to a specific essential molecule and the ill effects resulting from its absence. This might be the most difficult kind of damage to detect, since such a substance would have to be present in very small quantities to be depleted by small doses of ultraviolet irradiation. The fact that the substance need not be the primary absorber complicates attempts to characterize it from the action spectrum for damage.

Repair in this case implies resynthesis, or the provision of an alternate pathway, since by gross damage we mean damage beyond simple repair. While this is possible in isolated cases, it seems unlikely that it could result in a phenomenon as widespread as photoreactivation is found to be. Furthermore, the possibility that alternate pathways are provided is unlikely, in view of the fact that damaged and reactivated species show the same radiation sensitivity as the original material.

2) Minor damage to a substance essential to normal metabolism. Photoreactivation could then imply repair of the damage. The most likely possibilities would appear to be photoisomerization, photooxidation, or photoreduction. The first of these might result, for instance, from the breakage of a number of adjacent hydrogen bonds in a nucleic acid or protein with reorientation of rotatable groups. It is not easy to see what simple pathway there could be for photoreactivation of this effect.

Perhaps the most plausible kind of repairable damage in this category would be damage by photooxidation, followed by photoreduction in the reactivation step. So far as I am aware, no such general mechanism has been previously considered. However, we know that the in vitro irradiation of aqueous systems with ultraviolet in the 2500-angstrom range often results in photooxidation, with the formation of peroxide or some other unpleasantly reactive species.

We know also that less highly excited molecules can (via their triplet states) act as oxygen scavengers, and recent work has shown that triplet-state molecules also react readily with peroxides and with molecules possessing unpaired electrons.

Thus, damage and photoreactivation might involve the initial formation of a peroxide or other oxidized form by an oxygen reaction, followed by reduction in a direct reaction with triplet-state molecules formed by the action of visible light.

This is an attractive hypothesis and might account for the generality of the process and the apparent diversity in the photoreactivating substance. On this model, the substance involved is simply whatever pigment (or other conjugated molecule with a fairly low-lying absorption band) has a suitable triplet state.

3) Photodamage in which the presence of the damaged molecule (rather

than absence of the normal one) is the undesirable effect. This hypothesis might avoid the necessity of postulating that the sensitive substance is a very minor cell constituent and still fit a good many of the known facts.

Since x-ray or other high-energy damage is not photoreactivable, it is unlikely that the undesirable species is an unusual ion or simple free-radical, since such species are produced in abundance at high energies. Again we find the hypothesis attractive that some photooxidized species is most probably formed, and may be removed by reaction with a molecule excited by visible light, probably after the latter has gone over to the triplet state.

In this triplet-state hypothesis we cannot entirely rule out the reverse process—that the triplet molecule first reacts with oxygen to form a labile oxygen carrier able to oxidize a molecule photoreduced in the initial ultraviolet irradiation. On the whole, this process seems less probable than that suggested first, however. If it did actually occur, it seems likely that the peak in the action spectrum for photoreactivation might correspond to a peak for photodynamic killing. Although such a correlation has not been seriously investigated and therefore cannot be ruled out, there is no evidence to suggest that it occurs. This suggests that any triplet state-oxygen adduct formed is a harmless one and encourages us in the belief that the photoreactivation adduct is also harmless and that the triplet state may thus act as a scavenger of lethal oxidation products or radicals.

Damage by high-energy radiation. I do not propose to discuss this area in any detail, since, although an immense amount of work has been done, only the most general statements can be made about mechanism. The situation at present seems to be that we cannot even say with confidence what the major damaging effects are. The chromosomal apparatus, being delicate and fairly easily observed, has received a great deal of attention. If we try to visualize the outcome of the passage of a densely ionizing particle through an ordered, hydrogen-bonded macromolecule possessing a number of easily oxidized, reduced, or fragmented groups, the situation is understandably complex. We may expect radical recombinations of the fragmented backbone to cause gross changes which may be reflected at the microscopic level. Secondary electron bombardment will cause electron ejection and consequent oxidation of groups unaffected by normal oxidizing agents. Configuration changes or denaturation may occur because of gross breakage of many adjacent hydrogen bonds. Reducible groups will act as traps for slow electrons, and the delicate charge balance of the molecule will be severely upset. Very reactive free-radical fragments will attack undamaged species, producing macromolecules not normally encountered biologically. Superposed on these direct effects, the well-investigated "indirect effects" of attack by hydroxyl radicals (H_2O_2 and so on) formed from water will complicate the situation. Finally, dangerous autolytic processes may be induced by cell membrane discharge resulting from the unusual abundance of ions and electrons.

Many of these complex phenomena have been reported as identifiable results of radiation damage. It seems unlikely that we will obtain a very clear picture of their relative importance for a very long time, but meanwhile work of the kind that is in progress does focus attention on the biophysical aspects of biological survival and may well prove fruitful in the study of normal as well as of abnormal cell processes.

References and Notes

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Hans Friedrich Jensen, Biochemist

Hans Friedrich Jensen was born in Hamburg, Germany, on 5 July 1896, the son of Jens and Emmelie Jensen. He received his early education in Hamburg and served in the German army during World War I. It was not until 1921 that he received his Ph.D. in organic chemistry at Göttingen, working under the direction of Adolph Windaus. From 1921 to 1922 he served as assistant at the Kaiser Wilhelm Institut für Chemie, Berlin, and from 1922 to 1923, as assistant at the University of Göttingen. He came to this country in 1924 and served as assistant professor of chemistry at the University of Louisville until 1927. His dissertation at Göttingen had been concerned with some aspects of the chemistry of the imidazoles, and this interest in heterocyclic chemistry continued during the period of his stay at Louisville.

In 1927 Jensen joined Abel's group in the department of pharmacology at the Johns Hopkins School of Medicine immediately after Abel and Geiling had