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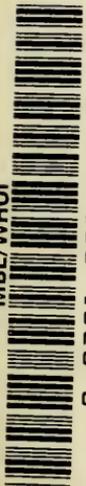


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RADIATION BIOLOGY

*VOLUME III*



# RADIATION BIOLOGY

VOLUME III: VISIBLE AND NEAR-VISIBLE LIGHT

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RADIATION BIOLOGY, VOLUME III

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## PREFACE

The field of visible and near-visible light embraces a part of the spectrum somewhat artificially separated from the radiations discussed in the first two volumes of "Radiation Biology," i.e., ultraviolet and high-energy. Since the relation between the visible (and near-visible) and ultraviolet parts of the spectrum is rather close, any separation of the two is arbitrary. This volume may be used as a separate work. But, since treatment of material overlaps among the three volumes, more benefit can be derived if all are read as a unit. Although rapid progress in many aspects of photobiology has been made in the last three years, i.e., after the manuscripts were completed, the basic information in this book is of greatest importance.

Special thanks belong to Dr. Sterling B. Hendricks, who carried great responsibility in assembling the manuscripts for this volume.

ALEXANDER HOLLAENDER





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RADIATION BIOLOGY

*VOLUME III*



## CHAPTER 1

# Energy Exchange in Photoreactions<sup>1</sup>

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*Introduction: Scope of this chapter—Potential energy and light absorption—Black-body radiation—Potential-energy diagrams. Internal conversion of energy: Conditions for intersection of potential-energy surfaces—Types of internal-conversion processes—Conjugated molecules. Energy transfer during adiabatic collisions: Absolute theory of collisions—Energy transfer involving rotational degrees of freedom—Adiabatic processes of vibrational-energy exchange—Collisions of polyatomic molecules. Energy exchange in diabatic processes: Types of diabatic processes—The quenching of fluorescence—Energy transfer between electronic degrees of freedom—Quenching with vibrational-energy transfer. Chemiluminescence. Energy transfer in biological reactions. References.*

### 1. INTRODUCTION

#### 1-1. SCOPE OF THIS CHAPTER

The photoreactions of everyday importance to life are associated with wave lengths of electromagnetic radiation lying in the region from about 2000 to 10,000 Å. At short wave lengths, absorption of radiation by most molecular species will result in permanent, nonuseful decomposition. The shorter wave lengths are largely removed from the sun's radiation by atmospheric absorption. At long wave lengths, energy will be taken up by single vibrational, rotational, or translational degrees of freedom and will be rapidly dissipated, not to produce chemical reaction, but in the trivial process of adding to the thermal energy of the system. Common processes in the ultraviolet are not likely to be apparent, because radiation in this region is not visible. A few important biological processes depend on radiation in this region. For instance, the formation of vitamin D from its provitamin is a well-publicized process. The rate of mutation production may also depend on the damaging effect of ultraviolet radiation on genetic structure. In general, the more colorful processes caused by visible radiation have attracted more biological attention. Vision itself, photosynthesis, growth mechanisms in plants, and numer-

<sup>1</sup> Contribution from the Laboratory for the Study of Photosynthetic Processes, supported by the Atomic Energy Commission.

<sup>2</sup> Now at School of Chemistry, University of Minnesota.

ous other common examples of processes depending on specific effects of visible light have provoked research interest for many years. These phenomena are gradually becoming understood, largely in terms of the simpler photochemical systems, which can be duplicated in the laboratory.

The general principles of the energy-exchange processes that occur in chemical and physical systems were made understandable during the period 1925-1942. Photoreactions provide a special case only in so far as the magnitude of the energies involved is greater than ordinary thermal energies and in so far as special transfer of electronic excitation energy may be important. There are many important gaps in our knowledge

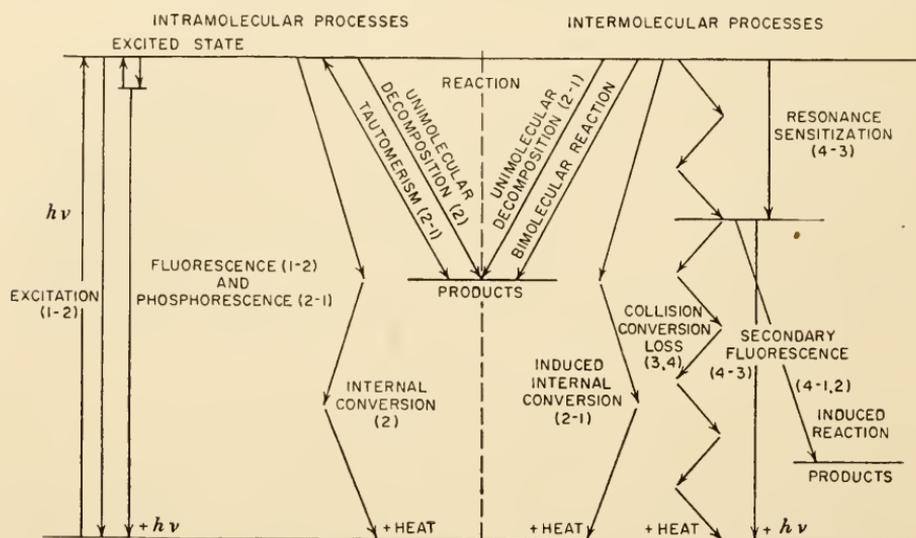


Fig. 1-1. Processes that follow the absorption of radiant energy. Vertical lines denote radiation processes. Diagonal lines indicate internal conversion or the exchange of energy among degrees of freedom other than electronic. The number following each term is that of the section or subsection in which that phenomenon is primarily discussed.

of energy-transfer processes. Nevertheless there exists a voluminous literature on the subject, so that we can hope to abstract only a few of the major areas of the field here. General discussions of energy-transfer processes have been given by Bethe and Teller (1940), Oldenberg and Frost (1937), Zener (1935), and Franck and Livingston (1949).

In Fig. 1-1 the various possible sequences of steps following light absorption or preceding luminescence emission are diagramed. Diagonal steps represent processes that occur without absorption or emission of light. The number that follows each term refers to the section of this chapter which deals with the phenomenon. No claim is made for the completeness of coverage of any topic. However, the years have shown that the most convenient and lucid visualization of kinetic systems is in terms of potential-energy surfaces on which each point represents the potential energy for a distinct configuration of the atoms of one or several

molecules. An attempt has been made to unify most phases of energy transfer in terms of a single type of potential-energy diagram.

## 1-2. POTENTIAL ENERGY AND LIGHT ABSORPTION

The potential energy of any collection of atoms can be diagrammed in a hyperspace with dimensionality equivalent to the number of independent interatomic distances plus one additional coordinate for the values of the energy. It is immaterial whether the assembly of atoms is stable or unstable. In the simplest case of a stable diatomic molecule, two dimensions are required: the interatomic distance and the potential energy. The relation of these two variables is satisfactorily represented by a Morse function (Morse, 1929)

$$E = D'(e^{-2a(r-r_0)} - 2e^{-a(r-r_0)}), \quad (1-1)$$

where the symbols have the meanings shown in Fig. 1-2. In this figure there are two regions of stable atomic configuration. One occurs at infinite separation lying to the right; the other, near the bottom of the potential well at the equilibrium separation  $r_0$ . The two situations are separated by the potential energy  $D$ , equal to  $D'$  minus the zero-point energy  $\frac{1}{2}h\nu_0$ , where  $\nu_0$  is the fundamental frequency of oscillation equal to  $\frac{1}{\pi 2} \left( \frac{f}{\mu} \right)^{1/2}$ , in which  $\mu$  is the reduced mass of the molecule and  $f$  is the force constant existing between the atoms. Solutions of the wave equation yield the allowed vibrational states, which are integral multiples of the fundamental frequency. Each horizontal line cuts the potential well at the maximum and minimum interatomic distances through which the atoms may pass in the steady oscillation allowed for the given vibrational state. At these two points the energy is primarily potential, and the atoms will move most slowly relative to each other in the region of these points. According to the Franck-Condon principle (Franck, 1926a), electronic transitions will occur most frequently in these regions, and indeed so rapidly ( $10^{-15}$  sec) that there is no appreciable change in the interatomic configuration, which can be altered only in the characteristic time of  $10^{-12}$ – $10^{-14}$  sec. Electronic transitions, under these conditions, are said to be adiabatic. Accordingly paths 1 and 2 in the two-dimen-

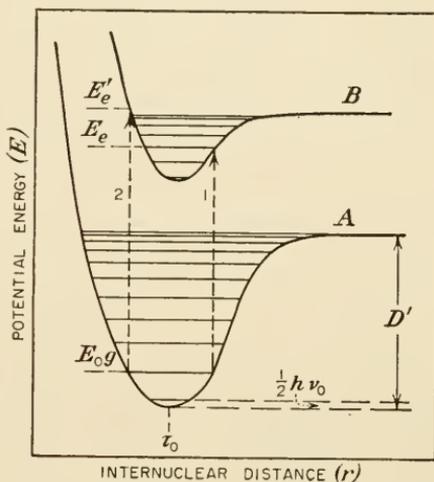


FIG. 1-2. Franck curves of potential energy for the ground and first electronically excited states of a diatomic molecule. The symbols include those of the Morse function describing the ground state.

sional potential-energy diagram of Fig. 1-2 represent a stable and an unstable transition occurring when the molecule is exposed to electromagnetic radiation with frequency given by  $\nu = (E_e - E_g)/h$  and  $\nu = (E'_e - E_g)/h$ , respectively,  $h$  being Planck's constant. The primary process of photoexcitation takes place if certain selection rules derivable from quantum mechanics are obeyed. The most common form takes place with probabilities related to the difference in dipole moments existing between the two states of the molecule and to the intensity of radiation in the field. The transition probability coefficients derived by Einstein are functions only of the molecule.<sup>3</sup> For the transition from a ground state with wave function  $\psi_g$  to an excited state  $\psi_e$ , the probability that a radiation field of unit intensity will cause a transition to take place is

$$B_{g \rightarrow e} = \frac{8\pi^3}{3h^2} |R_{ge}|^2. \quad (1-2)$$

$|R_{ge}|$  is the matrix element of the dipole moment of all the electrons in the molecule between the two states; i.e.,

$$R_{ge} = \int_{\tau=0}^{\tau=\infty} \psi_g^* \epsilon \sum_j r_j \psi_e d\tau$$

in which  $\epsilon$  is the charge on the electron,  $r_j$  is the radius vector of the  $j$ th electron, and the asterisk means a complete conjugate. In molecules in which dipole coupling alone occurs, as here discussed, only the extra shell electrons need be considered. The same situation obtains for single atomic species, and the equations are identical. If the excitation energy is not first lost by dissipation into internal degrees of freedom or in collisions with other molecules, fluorescence will occur (path 3, Fig. 1-3)

either as a first-order process independent of the radiation field or by the induced effect of this field. The corresponding transition coefficients are

$$A_{e \rightarrow g} = \frac{64\pi^4 \nu^3}{3c^3 h} |R_{eg}|^2 \quad \text{spontaneous}; \quad (1-3)$$

$$B_{e \rightarrow g} = \frac{8\pi^3}{3h^2} |R_{eg}|^2 \quad \text{induced}. \quad (1-4)$$

<sup>3</sup> For a discussion of the equations in this section, see any treatise on quantum mechanics, for instance, Pauling and Wilson (1935) and Eyring *et al.* (1944).

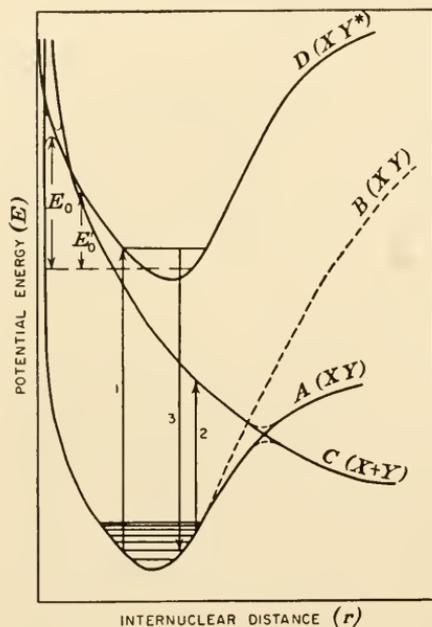


FIG. 1-3. Ground and excited electronic states of the diatomic molecule  $XY$ .  $D$  is a stable excited electronic state,  $C$  an unstable excited electronic state.

## 1-3. BLACK-BODY RADIATION

At ordinary temperatures, induced emission is small compared with spontaneous emission. Black-body radiation has an appreciable intensity only in the far infrared at ordinary temperatures. In each second there will be a small fraction  $A_{eg}e^{-E/RT}$  of molecules emitting. Taking  $A_{eg} = 10^7$  and  $E = 35,200$  (corresponding to red light of 6000 Å), we obtain  $3 \times 10^{-18}$  for the fraction of molecules emitting per second. By the principle of detailed balancing, this is likewise the fraction of molecules activated per second by black-body radiation. The rate for this same excitation by collision is  $\frac{kT}{k} e^{-(E+E_0)/RT}$ , or faster than black-body excitation by a factor of  $6 \times 10^5$ , providing the added activation energy  $E_0$  is zero.

The principle of detailed balancing (or microscopic reversibility) requires at equilibrium that the number of reactions in the forward direction on any reaction path equal the number in the backward direction. Thus quenching and excitation by collision balance according to the equation

$$k_{eg}N_tN_e = k_{ge}N_tN_g, \quad (1-5)$$

in which  $k_{eg}$  is the rate constant for quenching of radiation from state  $e$ ,  $k_{ge}$  is the rate constant for excitation from  $g$  to  $e$ ,  $N_t$  is the number of molecules acting as quenchers and exciters by thermal collision,  $N_e$  is the number of excited molecules, and  $N_g$  is the number of molecules in the ground state. Similarly

$$\rho(\nu)B_{ge} = \rho(\nu)B_{eg} + A_{eg}, \quad [\rho(\nu)B_{eg} \ll A_{eg}] \quad (1-6)$$

in which  $\rho(\nu)$  is the intensity of black-body radiation of frequency  $\nu$ . The fraction of molecules quenched in a simple case is

$$Q = \frac{k_{eg}N_t}{A_{eg} + k_{eg}N_t} \quad (1-7)$$

and can be determined from experiment. For levels where  $Q$  approaches zero, clearly the radiation hypothesis of activation (i.e., black-body activation outruns collisional activation; Kassel, 1932, p. 313) is not at all a dead issue, since here emission outruns quenching, and it follows that for the reverse process activation by black-body radiation will correspondingly outrun activation by collision.

## 1-4. POTENTIAL-ENERGY DIAGRAMS

The two-dimensional potential-energy diagrams thus far employed are strictly applicable only to diatomic molecules. Little loss in generality is incurred if the curves are used to represent cross sections through the many-dimensional surfaces of polyatomic molecules. In these cases the

abscissa is any nuclear separation under discussion. Excited states for polyatomic molecules, represented on the same plot, will only infrequently have the same degree of freedom involved in any reaction process as does the ground state. To avoid complexity in presentation, we must generally ignore the latter fact. It is unavoidable that any visualizable simplification for the very complicated potential-energy situations that occur when there are more than two atomic separations is unsatisfactory. It will be found that these surfaces, represented as curves in two dimensions or contour maps of three dimensions, are inadequate for certain discussions. Unfortunately no more satisfactory alternative method for exposition is available.

The potential surfaces for diatomic molecules are widely separated along the energy coordinate. There will be, consequently, but a few excited states, if any, in the limit of photoenergies under consideration (2000–10,000 Å;  $1 \times 10^4$ – $5 \times 10^4$  cm<sup>-1</sup>; 28–142 kcal/einstein; 1.2–6.2 eV). Triatomic species have more closely spaced electronic levels, and generally the number of levels increases and their spacing decreases as the molecules increase in size and complexity. Any saturated organic molecule of more than three atoms will have a very dense distribution of electronic levels. If there are  $n$  bonding electrons in the molecule, there will be  $2^n$  eigenfunctions corresponding to the lowest states of the separated atoms. When the atoms are combined, this number is preserved, though not all the functions will correspond to different energies. The number of different surfaces is  $n!/(n!/2!)^2 \approx n^{-1/2}2^n$ . For most organic molecules this is a large number, yet states due to excited electrons have not been considered. The total fraction of states lying within 150 kcal, or 6.5 eV, of the ground state, though small, is still a large number, so that there is practically a continuum of states. The density of crossing points, i.e., points of intersection of surfaces, will be high, and it becomes apparent that precise treatment of the structure of energy-transfer processes for these molecules is very complicated. A quantum-mechanical treatment of a small protein molecule (molecular weight 50,000), for example, one composed entirely of leucine, according to present simplified methods would require consideration of something like

$$17,200^{-1/2} \times 2^{17201} = 10^{5175}$$

independent states—impossible complexity from a detailed point of view.

For a triatomic molecule such as N<sub>2</sub>O, there are eight valence electrons. A three-dimensional contour map of the type shown in Fig. 1-4 can be used to display each potential-energy surface. Two internuclear separations are required to describe the potential energy (plotted along the axis normal to the surface of the page) if the bending vibration is neglected, as it may be for convenience. Inclusion of this bending vibration would require an additional coordinate. The contour map of Fig. 1-4 is

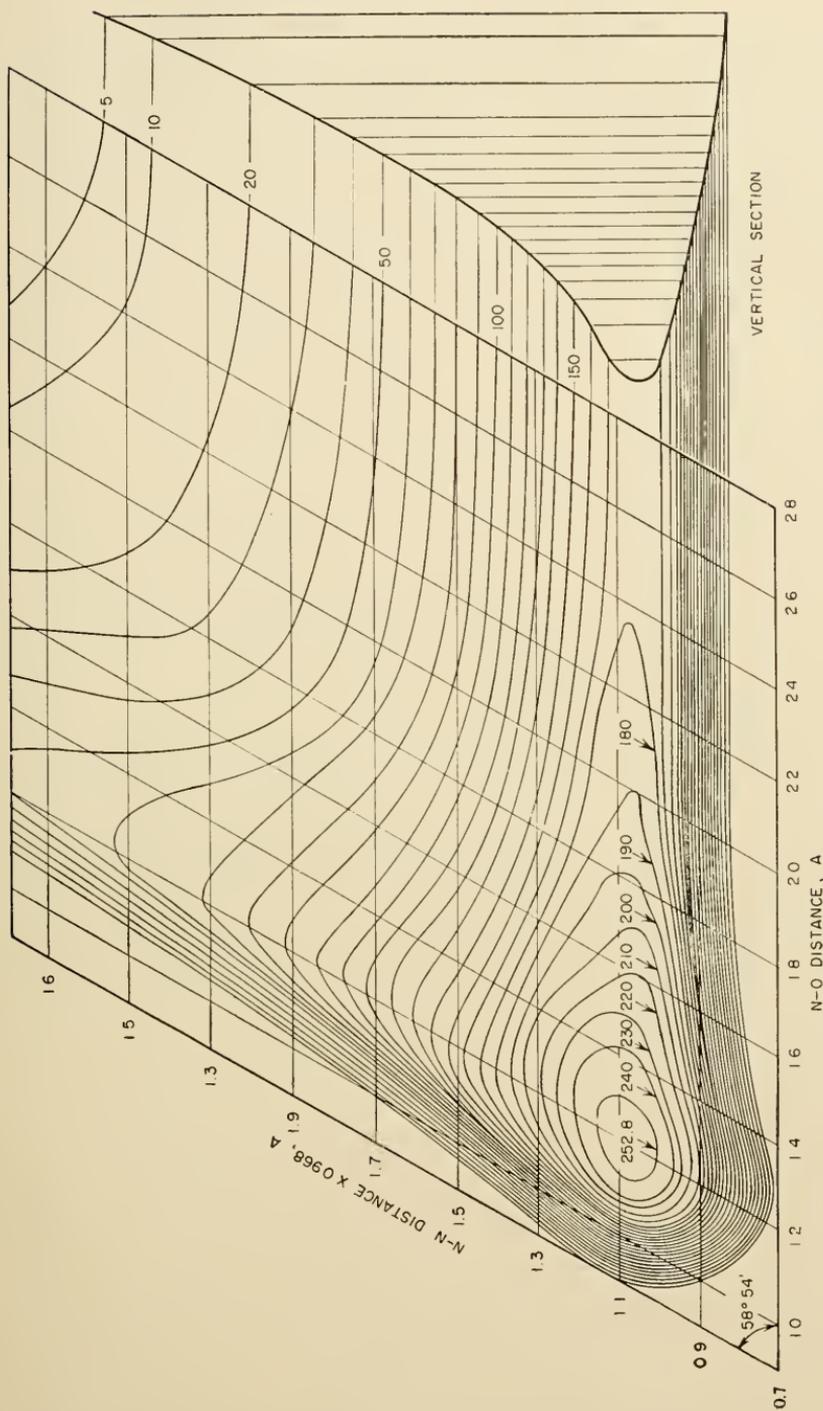
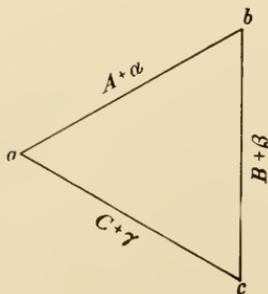


FIG. 1-4. Potential-energy surface for the reaction  $N_2O(^1\Sigma) \rightarrow N_2(^1\Sigma) + O(^1D)$  calculated for linear approach. This surface is typical of those for stable triatomic molecules. (Stearn and Eyring, 1935.)

typical of such plots for stable molecules. Increasing numbers of atoms in a molecule require multidimensional plots in which there is a valley of relatively low potential energy corresponding to great extension in each bond direction. A minimum, two valleys, and a plateau are seen to exist in Fig. 1-4. One of the valleys represents  $N_2 + O$ ; another,  $NO + N$ . Both lie off the figure out along the two axes shown. The least stable situation is the plateau at the upper right, and it represents complete separation into the atoms. The region of particular interest is near the origin. A vertical cross section parallel to either axis through the center of this region would look like the lower curve of Fig. 1-2, as seen to the right of Fig. 1-4. Excited states would lie above the one shown in Fig. 1-4. All potential surfaces can be approximately calculated by the "semiempirical" method of Eyring and Polanyi.<sup>4</sup> For a number of small molecules, very accurate potential relations have been calculated (for ground states) by detailed analysis of infrared spectra in much the same way that the dissociation energies for Morse functions are determined (Herzberg, 1945). Few excited states have been studied in this way, and only a very few molecules containing more than four atoms have been thus studied. The details of excited surfaces in the neighborhood of their potential minima have been calculated from spectra for only a limited number of molecules.

The semiempirical method is quantitatively unsatisfactory in most cases; yet it remains the only way to secure potential-energy information about complex molecules and for the calculation of absolute reaction velocities. It should be mentioned that energy transfer in and among molecules or atoms will be understood in terms of qualitative theories for many years to come. The trouble is the same that has beset all precise quantum-mechanical calculations, namely, mathematical complexity. Qualitative or, at best, semiquantitative information has, however, been remarkably satisfactory in advancing our knowledge of physical and chemical processes.

Consider a generalized triatomic molecule as pictured here:



<sup>4</sup> Detailed treatments of the "semiempirical" method, potential surfaces, and the  $N_2O$  system, in particular, are given by Glasstone *et al.* (1941, p. 337).

The species *abc* has three valence electrons, which can be considered to interact in pairs. In terms of the formalism of modern quantum mechanics, the interactions are of two types: (1) coulombic, due to electrical interaction of the charged bodies, nuclei, and electrons involved, and (2) exchange, due to the indistinguishability of electrons. Energies resulting from the latter ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) have no classical analogy but provide most of the stabilizing energy of the molecule. In fact, the coulombic energies  $A$ ,  $B$ ,  $C$  can usually be approximated as a small fixed fraction of their respective exchange energies (about 14 per cent). The total energy of the system of three atoms is given by a formula of London (1929a,b):

$$E = A + B + C - \{ \frac{1}{2} [ (\alpha - \beta)^2 + (\beta - \gamma)^2 + (\gamma - \alpha)^2 ] \}^{1/2} \quad (1-8)$$

Each total interaction, i.e.,  $A + \alpha$ , etc., can be approximated by a Morse function, with constants for the pair of atoms determined from spectra. If the approximation with regard to coulombic energy is made, it is then relatively easy, though tedious, to evaluate the total potential energy for every separation of the atoms. Further complications arise in excited states because of the additional information needed for the new Morse functions, but when this is known, the excited-state potential-energy surfaces can be calculated in the same manner. In this way any desired potential-energy plot can be obtained in approximate form.

A linear triatomic molecule like  $N_2O$  can be graphed in three dimensions if the bending vibrations are neglected. The general case treated above requires more,  $3n - 6 + 1$ , for the general nonlinear molecule and one more for linear molecules.

It is now possible to consider the various processes which result from photoexcitation or which produce radiation from chemical energy. Potential-energy surfaces play a central role in such a discussion for the following reason: A random choice of coordinates to represent the geometrical situation that exists for a molecule at any instant will usually lead to a complicated expression for potential and kinetic energies in terms of these coordinates. It is possible, however, by taking suitable combinations of any such random set of coordinates to form another set containing the same number of independent coordinates, but one in which the energy expressions reduce to sums of terms in the single coordinates. That is, cross terms in two or more variables are eliminated so that motions in these "normal" coordinates, which may be extremely complicated visually, can be treated as independent of all other motions in other normal coordinates. In linear triatomic molecules, representation of the normal coordinates can be achieved by a simple method involving only the change in angle formed by the axes and the relative magnitude of units on the two axes. Normalization in Fig. 1-4 was obtained by casting the axes at an angle less than  $90^\circ$ . A useful property of potential-energy surfaces is the fact that after normalization the frictionless move-

ment of a mass particle, which is originally given the proper total energy, approximates<sup>5</sup> the changes of geometry in time for systems of a few atoms. The movie record of such a process as, for instance, the oscillation of a ball in the potential well of the stable triatomic molecule would yield the approximate history of energy transfer for the process in question. We shall make use of this property.

The potential-energy diagrams thus far considered have been for chemically stable systems (for example, Fig. 1-4). The method for their calculation, as previously outlined, applies equally well to unstable systems and has, indeed, received its principal development in this application. The region of the surfaces of these diagrams corresponding to

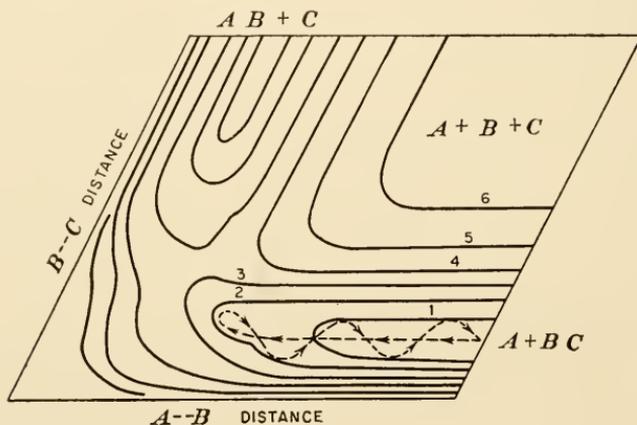


FIG. 1-5. Typical contour map of potential energy for the simple replacement reaction  $A + BC \rightarrow AB + C$ . The dotted line is a collision path in which translational energy is converted into vibrational energy.

small distances between the stable entities involved is now characterized by a potential barrier rather than a well. Figure 1-5 is an example of such a system. The dotted path of a rolling ball describes the redistribution of energy that takes place between the colliding molecule and the diatomic species  $BC$  in a single collision. An inelastic collision takes place, in that part of the relative kinetic energy of translation of the two molecules is transferred to vibrational energy of  $BC$ , as indicated by the increase in oscillatory motion of the system across the potential trough. Had the original translational energy been high, the system would have crossed the potential barrier to produce a simple displacement reaction (the figure represents collision along the longitudinal axis of the diatomic molecule only). For present purposes the energy is taken as less than the value necessary to produce reaction. In contrast to the situation for stable entities, systems containing complex molecules are not so

<sup>5</sup> Actually, if the vertical motion is kept small by an appropriate scaling factor, this approximation can be made excellent.

readily represented in terms of the three-dimensional contour map, one cross section of which is shown in Fig. 1-5. However, the various situations can be investigated in a qualitative fashion in terms of the simpler diagrams.

## 2. INTERNAL CONVERSION OF ENERGY

### 2-1. CONDITIONS FOR INTERSECTION OF POTENTIAL-ENERGY SURFACES

Internal conversion is defined as the exchange of energy between electronic and vibrational degrees of freedom within a single molecule (Teller, 1937; Franck and Livingston, 1941). The essential distinction from other energy-exchange processes is that it involves the crossing of potential-energy surfaces for different electronic states, as shown in Fig. 1-3. Molecular electronic states are either stable or unstable. If excitation is to an unstable state *C* (Fig. 1-3, path 1), the molecule, in coming to equilibrium in the new electric field of the electrons, undergoes a separation of its parts, and the excess of energy above that required to form the products appears in external degrees of freedom. A part of the excess may pass through the vibrational degrees of freedom in so doing. When the state is stable, several situations can occur, depending on the amount and type of crossing possible from this state. If there is no crossing point available except with high activation energy, as shown in Fig. 1-3, fluorescence will occur with high probability via path 3. Increasing temperature increases the rate of crossing the barrier with height  $E_0$  or  $E'_0$ , so that its effect will be one of quenching, i.e., diminution of fluorescence. This is a common observation for most fluorescing substances. In Fig. 1-3 quenching returns the molecule to its ground electronic state *A*, with energy greater than the dissociation value. Had the ground state been represented by Fig. 1-5, *B*, no dissociation could result, and the electronic excitation energy would be completely converted into vibrational energy of the ground state, subsequently to be lost as heat.

Internal conversion need not be to the ground state, but to any other state of higher or lower energy whose surface crosses the surface representing the initially excited state. Rearrangement of energy by crossing of surfaces is radiationless and hence subject to weaker restrictions than apply to optical transitions. Crossing may occur between levels with different multiplicity,<sup>6</sup> as shown in Fig. 1-6. According to Lewis and Kasha (1944), this is an explanation of phosphorescence; i.e., radiation is

<sup>6</sup>The term "multiplicity" refers to the quantum number of the resultant spin angular momentum for all the atoms of the molecule. If the over-all spin vector has an associated quantum number  $S$ , the multiplicity is  $2S + 1$ , and that is the number of components of the spectral lines corresponding to the particular state. It bears a direct relation to geometrical symmetry. Optical transitions between states of different multiplicity are allowed only with very low probability.

delayed by the prohibition of radiational transitions between states of different multiplicity. Either the transition probability to the ground state will be low (Fig. 1-6, path 2), or an activation energy  $E_0$  is required to restore the system to the original surface from which radiation is allowed (Fig. 1-6, path 3). Temperature dependences will frequently distinguish which situation exists. The phosphorescence of anthracene, biphenyl, and thiobenzophenone appear to be thus explained. On the other hand, the state to which crossing occurs may belong to a tautomer of the original molecule (Franck and Livingston, 1941). For example,

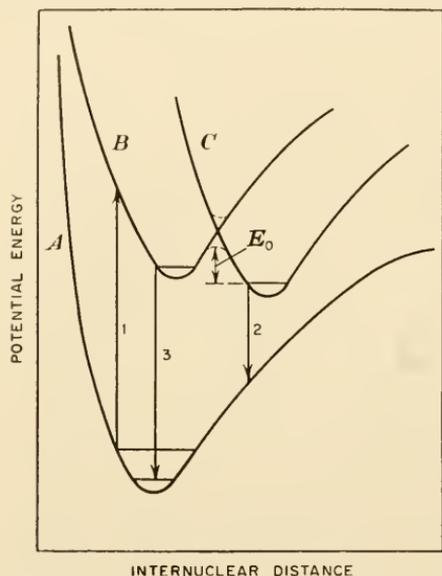


FIG. 1-6. Potential-energy curves illustrating internal conversion to a metastable state  $C$ , triplet, or other tautomer.

phosphorescence can be an important final result, as Franck and Livingston (1941) have proposed. The phosphorescence of tryptaflavine under special conditions has been explained in this way (Kautsky *et al.*, 1933; Weiss, 1935). Internal rearrangement of nuclei is probably important in many photoinduced reactions. Several of the reactions of chlorophyll *in vitro* have been satisfactorily explained in this way, but the interpretations are not unequivocal.

Generally speaking, the consideration of single excited levels greatly oversimplifies the picture. In Sect. 1-4, the number of surfaces for  $n$  unexcited atoms was given as  $n!/(n!/2!)^2$ . In the absence of symmetry in the molecule, this is the number of different energy levels. If  $n$  is 16, there will be  $3 \times 10^4$  potential-energy surfaces corresponding to unexcited atoms. If all these states lie within 100 ev (2300 kcal) of the ground state, the levels will be about 3.3 mv, or 76 cal, apart. Very nearly a

one or more hydrogen atoms of chlorophyll may take new positions during the crossing process to form a new chemical species, though modern evidence favors a triplet metastable state (Franck, 1951). If the ground state of the new species lies higher than the ground state of the original form, some of the excitation energy is stored as the additional free energy of formation of the tautomer. The transition from  $C$  to  $A$  may be forbidden because (1) it involves a change in multiplicity or (2) curve  $C$  may actually cross  $A$  and so be the lowest surface for the configuration corresponding to the minimum in  $C$ . When reversion to the original molecular form in its excited state is restricted by an appreciable activation energy, phosphorescence can be an important final result, as Franck and Livingston (1941) have proposed.

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continuum of surfaces exists with a high density of crossing points. Although it is correct to talk of single excited states for diatomic molecules, such simplification may be incorrect for polyatomic molecules even when the region of consideration is the very lowest on the energy scale with depth no greater than perhaps 8 eV, as is the case in the present discussion. At least at the top of this small band the density of levels is high for molecules of low symmetry, so that crossing becomes important and the original excited state will have a high probability of undergoing a series of rapid changes to other excited states. In saturated molecules each state may be associated with a single bond, at least to a fair first approximation. By repeated crossing of potential-energy surfaces, electronic potential energy can be passed around among stable states from bond to bond. If there is no mechanism with sufficiently low activation energy whereby the potential energy of stable excited electronic states can be converted into vibrational kinetic energy, dissociation will not occur even though the excitation energy exceeds that required for rupture of a bond. Benzene, for instance, fluoresces with high yield in the region 2800–2200 Å (Sponer and Teller, 1941), whereas the einstein for these wave lengths has the values 120–128 kcal, which are more than adequate to cause the reaction (Roberts and Skinner, 1949)



Dissociation in stable states can be caused only by localization of energy equivalent to chemical reaction as vibrational energy in a single bond or vibrational mode of motion. This excitation energy may be used as free energy of activation or as thermodynamic energy to raise the products to a higher level of free energy. Photoisomerizations, such as those of maleic and fumaric acids (Warburg, 1919) and of 1,2-dichloroethylene (Bonino and Brüll, 1929), are good examples of photoprocesses in which the energy is used as activation energy. Theoretical treatment of this type of photoprocess has been given by Olson (1931, 1933).

The crossing of potential surfaces occurs when the nuclei of a molecule achieve a configuration identical with that of another electronic state, as represented in Fig. 1-7. This figure is drawn in a different way from that previously employed to break the combination of potential surfaces for the single electronic states into

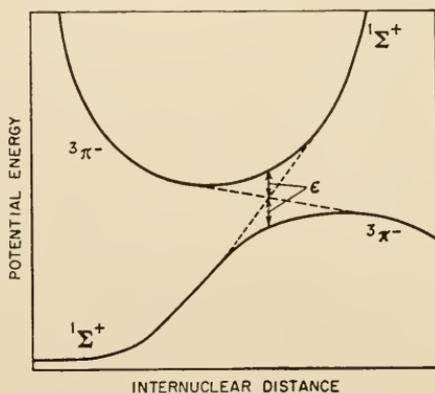


FIG. 1-7. Detailed drawing of a crossing point between typical potential-energy surfaces. Dashed lines are usually drawn solid in other figures.

upper- and lower-lying potential-energy surfaces independent of the electronic state.

According to Landau (1932) and Zener (1932), the probability of the change from one potential-energy surface of a diatomic molecule to another at a crossing point is given by the expression

$$p = e^{-4\pi^2\epsilon^2/hv|S_i - S_f|}, \quad (1-9)$$

in which  $2\epsilon$  is the smallest potential-energy difference existing between upper and lower curves;  $v$  represents the relative velocities of the nuclei as they pass through the crossing point, i.e.,  $dR/dt(0)$ , if  $R$  is the inter-nuclear distance and 0 refers to the crossing-point value of  $R$ ; and  $|S_i - S_f|$  is the absolute value of the difference between the quantities  $dE_i(R_0)/dR$  and  $dE_f(R_0)/dR$  if  $E_i$  and  $E_f$  are the potential energies on the initial and final surfaces. Teller (1937) has extended the treatment to polyatomic molecules (see also Kramers, 1940; Neumann and Wigner, 1929; and Wigner, 1927). When  $2\epsilon$  is large, as it is when the states are very similar and thus interact strongly to give a large resonance energy, Eq. (1-9) ceases to apply. However, it does indicate correctly that  $p$  will be exceedingly small. When the states change at the osculating point (Fig. 1-7) in spin quantum number or in symmetry or angular-momentum quantum number, or, indeed, if an electron is transferred from one atom or ion to another, crossing may occur with the probability given by Eq. (1-9). Only when quantum numbers do not change, or change in the ways required by optical selection rules, will the interaction  $\epsilon$  be large and crossing a poorly probable process. Thus, in general, systems of colliding molecules will demonstrate the same total values of each quantum number before and after collision. It is apparent that these rules will manifest themselves in a general predilection for the system to retain its original quantum numbers at crossing points. Such behavior has been especially predicted for the spin-angular-momentum quantum number and is called the Wigner (1927) spin-conversion rule.

Van Vleck (1932) and Zener (1933a) have proposed that external fields will increase the probability of crossing favoring the recoupling of nuclear spins. Turner (1930) observed a decrease of  $I_2$  fluorescences in a magnetic field. The presence of paramagnetic ions, for example,  $Fe^{++}$ ,  $Co^{++}$ , and  $Fe^{3+}$ , may act in a similar manner in quenching, since these ions are frequently very efficient quenching agents and are known to favor the interconversion of ortho- and parahydrogen. In Sect. 4-3 an alternative mechanism involving oxidation and reduction of the ions (Weiss, 1939b) is proposed as an explanation of their effectiveness in quenching. The theory of Zener predicts that electric fields will increase the probability of crossing between states of the same multiplicity. Field-intensity requirements are apparently too high to allow observation of the phenomenon.

Substances causing either type of induced internal conversion will not

generally receive much of the excess energy. In collision processes, on the other hand, there are no rigid restrictions on crossing phenomena, and the energy of both participants can be redistributed in any way consistent with the conservation of energy and momentum and quantum-mechanical restrictions on allowed energy levels.

Internal conversion may be favored by low field energies of neighboring molecules, especially of ions through their perturbing effect on energy levels (Stark and Zeeman effects) rather than through the less probable destruction of crossing restrictions mentioned previously. Crossing and resonance transfer processes greatly depend on the difference in energy  $2\epsilon$  between states. Perturbation due to neighboring molecules can produce better matching of electronic states, thus favoring more efficient crossing. Some additional useful papers dealing with the subject are Zener (1933a), Teller (1941), Spomer and Teller (1941), Herzberg and Teller (1933), Franck (1926b), Franck and Eucken (1933), Franck and Rabinowitch (1934), Nordheim (1926), Rosen (1933) and Hirschfelder and Wigner (1939).

So long as the separation of surfaces at a crossing point is not large, the presence of Planck's constant  $h$  as a factor in the exponential assures a high probability for the process once the crossing point is reached. Furthermore, if such points lie below the total potential energy of the system, crossing should occur rapidly, for in this case less than a total period of a stretching vibration of the nuclei will produce the crossing configuration. The characteristic periods for such motion are of the order of  $10^{-12}$ – $10^{-14}$  sec. Bending vibrations are considerably slower and probably not generally important in most crossing phenomena. Vibrations involving heavy nuclei will be slower. The time required for crossing along a reaction coordinate from one potential surface to another will vary within wide limits depending on the distribution of crossing points relative to the excitation-energy value and the number of degrees of vibrational freedom effective in the excited state. Crossing is a unimolecular reaction on excited surfaces and hence subject to the same treatment of energy redistribution to be given in Sect. 3-4 for vibrational degrees of freedom. The difference in energy between the crossing point and the lowest vibrational energy in the excited state will correspond to an activation energy for the unimolecular process (Fig. 1-6). Molecules that never fluoresce must be able to cross in less than about  $10^{-8}$  sec. The minimum time for crossing in most processes cannot be less than about  $10^{-14}$  sec, since this is the lower limit of vibrational times.

## 2-2. TYPES OF INTERNAL-CONVERSION PROCESSES

The probability of internal conversion from an excited state will depend on the kind of molecule under consideration. Saturated hydrocarbons lack symmetry and consequently have a very dense energy structure

above the first excited state. Many different excitation processes may correspond to the same energy, and crossing points are common. The spectra of such molecules begin in the far ultraviolet and are continuous, indicating dissociation (Noyes and Leighton, 1941, p. 327; Spomer and Teller, 1941). Saturated hydrocarbons probably do not fluoresce, and we may conclude that each absorption process is immediately followed by internal conversion whenever excitation is originally to stable states. The extra energy will be in part taken up by other molecules, but the larger amount will be concentrated in the vibrational degrees of freedom of the primary molecules. Dissociation and reaction from stable states can occur only if the energy becomes localized in suitable vibrations.

Saturated hydrocarbons have no single electrons that are especially loosely held; hence the short wave lengths required for excitation. Molecules containing halogen, oxygen, nitrogen, or metal atoms or double bonds usually show "optical electrons." These may be considered as belonging to a special part of the molecule. Many such molecules—for example, aldehydes, ketones, carboxylic acids, olefins, and amino and cyano compounds—show Rydberg-like spacing of levels in higher excitation energies, much like the familiar spectral distributions of atoms and attributable to a localization of excitation in a single atom or group (Spomer and Teller, 1941). Light absorption occurs at wave lengths in the near-ultraviolet or visible bands, and fluorescence is a common accompanying phenomenon, thus indicating poor crossing to the ground state. The onset of dissociation as wave length is shortened is frequently characterized by predissociation spectra (Sect. 4-1). Most organic molecules belong to this second class, i.e., those with groups containing localized electrons, but there is a third group which, though smaller, is of more importance in the photoinduced processes associated with living systems. These are the molecules in which there exist suitably spaced combinations of atoms or groups of atoms with optical electrons to allow migration of the electrons over large distances within the molecules. Molecules of this type are called "conjugated," meaning that they contain a conjugated system of electron-rich groups or atoms. Benzene is an important example of this class. The optical electrons associated with a single group can be described approximately in terms of the atomic orbitals, or at least group orbitals. For instance, the  $\pi$ -electrons forming the second electron pair of a nonconjugated double bond can be treated as belonging to this particular bond. In benzene, on the other hand, the  $\pi$ -electrons of the carbon atoms which form the three double bonds cannot be localized in either of the Kekulé structures. Instead, the two Kekulé clouds of allowed electron positions overlap. As a result, all six  $\pi$ -electrons can be pictured as moving through all overlapped clouds and must be treated as belonging to the molecule as a whole; i.e., they move

in molecular orbitals. In the higher excited states of benzene, a Rydberg-like series of levels is observed owing to the entire benzene molecule acting like a single atom for highly excited electrons (Sponer and Teller, 1941). Atoms with electrons over and above those required for the  $\sigma$  single bonds can substitute for double bonds to make the picture of conjugation quite general. Pyrrole, for instance, behaves somewhat like benzene, and the electronic correlation between thiophene and benzene is very close.

### 2-3. CONJUGATED MOLECULES

By far the largest number of molecules of importance in biological photochemistry are of the conjugated type. The list includes, among others, all the pigments of photosynthesis and flower coloration; riboflavin, which photosensitizes auxin destruction in the control of plant growth (Galston and Hand, 1949); and the pigments of all types of vision. Their distinct role can be attributed to five characteristics:

1. Low-lying excited states, frequently in the visible and hence useful for vision, photosynthesis, and the like, and at the same time not effective in destroying the molecule.

2. Stable excited states with poor crossing to the ground-state potential surface.

3. High electrical polarizabilities, i.e., loosely held electrons that favor interaction with other molecules.

4. Low ionization potentials.

5. Metallike conduction of electrons throughout the conjugated bond system.

These properties of conjugated molecules are worthy of further consideration:

1. The overlap of electron clouds, or, more accurately, of wave functions describing the positions for  $\pi$ -electrons, allows greater movement of the electrons in space and hence lower frequencies of sympathetic oscillation with the pulsating field of the incident radiation. Benzene has a long-wave-length cutoff at 2800 Å, but, as additional aryl rings are added, this is extended into the near ultraviolet and finally, with naphthacene, into the visible at 4500 Å (Sponer and Teller, 1941). The strong absorption of red light by chlorophyll is the result of extensive coupling of aromatic rings (pyrrole) conjugated to each other and to a magnesium atom introduced into their center. Addition of two hydrogen atoms in one pyrrole, strangely enough, improves conjugation, since bacteriochlorophyll, a photosynthetic pigment of bacteria, absorbs in the infrared at 8000 Å. Absorption in the visible and near infrared allows best use of that fraction of the sun's radiation which reaches the earth. It is, for instance, very important that the pigments of plants or animals not be destroyed in each primary process, since, in addition to the problem of

synthesis, such a course would divert the energy from useful function. Conjugated molecules fulfill the requirement because they absorb low-energy radiation inadequate for dissociation reactions.

2. Organic molecules that fluoresce in the visible region generally contain conjugated systems of bonds. Photosensitization efficiency for photoreactions of other molecules is associated with the ability to fluoresce, as would be expected, since fluorescence indicates the absence of internal conversion (Norrish, 1939; Hurd and Livingston, 1940). The probability of crossing to the ground surface from at least the first excited state is low, especially for planar, symmetrical, conjugated molecules that have widely spaced electronic states. The efficiency of crossing among

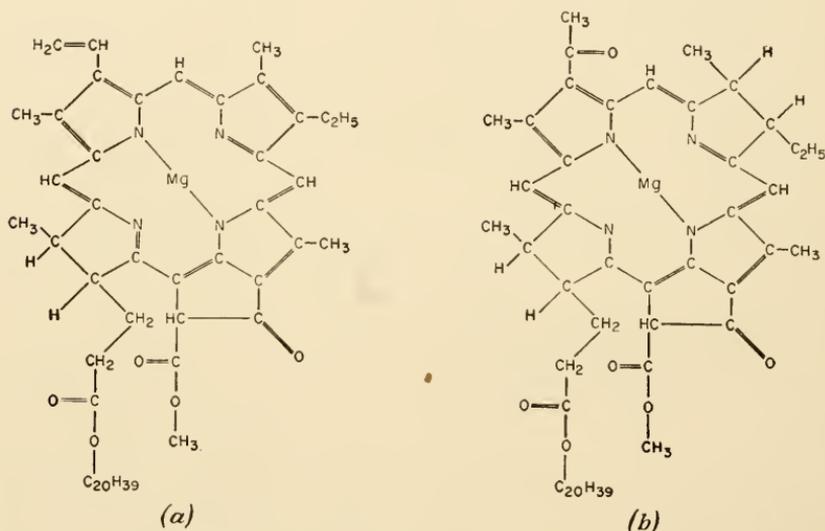


FIG. 1-8. (a) Chlorophyll a and (b) bacteriochlorophyll. (Fischer, 1940.)

upper levels will vary somewhat but is generally good, as witnessed by the common occurrence of long-wave-length fluorescence when excitation is at short wave lengths (benzene and chlorophyll are well-studied examples). Poor crossing to the ground surface can be roughly explained by the existence of "molecular" electrons rather than "atomic" electrons. The readjustment of electric fields which occurs in the crossing process is associated with changes in the equilibrium positions of most nuclei in the molecule rather than with the few nuclear position changes that occur in the crossing process for unconjugated molecules. In order for the configuration to reach the crossing point, a large number of internuclear distances must be simultaneously extended or shortened. The actual equilibrium C—C distance in benzene in its first symmetrical excited state is 0.037 Å larger than the ground-state distance (Craig, 1950). If a similar compression is required for the crossing between —C=C— states of an olefin and this occurs with probability  $p$ , all six conjugated

carbon-carbon bonds of benzene will have the appropriate length with probability  $p^6$ . This rough treatment indicates that the half-life of the olefin,  $\tau = 1/p$ , is perhaps less by a power of 6 than that of benzene at the same temperature. One may speak of a crossing region in the full potential-energy space of all coordinates rather than a crossing point. Crossing from excited states involving "atomic" electrons will have a considerable extension in potential-energy space, because a large fraction of the degrees of freedom are not sensitively involved in the crossing process and many nuclear distances need not be specified. These crossing regions will consequently be large by comparison with those for many conjugated molecules.

3.  $\pi$ -Electron bonds, being less stable than  $\sigma$ -electron bonds, can be deformed at lower energies. In other words, the  $\pi$ -electrons are readily displaced from their equilibrium positions by applied electric fields. This ease of polarizability has several useful features. It makes for large dipole differences between states and hence large transition probabilities. It makes association through van der Waals' dispersion forces, with molecules whose reactions are to be sensitized, a stronger and longer-lived phenomenon since these forces depend on the magnitude of the polarizability. It makes possible stronger electric-field interactions with neighboring molecules and thus increases the probability of energy transfer by resonance processes, as will be discussed in Sect. 4-3.

4. Conjugated systems generally have low ionization potentials. This fact is the result of the increased number of resonance structures that may be written for the ion of a conjugated molecule. According to a theory of Weiss (1939a), photosensitized oxidations and reductions as well as nonproductive quenching of fluorescence can be brought about by electron transfers to or from the excited molecule. Stabilization of ions should increase the efficiency of such a process. Further discussion of Weiss's theory is given in Sect. 4-3.

5. Because there exists a low-resistance path for  $\pi$ -electrons from one end of a conjugated system to the other, electron movements within the

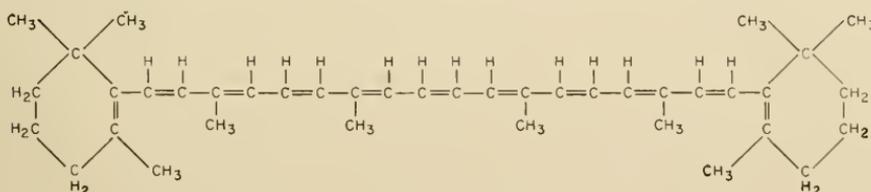


FIG. 1-9.  $\beta$ -Carotene.

system are very rapid. Speaking crudely, this has the effect that excitation energy in the form of an excited electron can be made available for reaction instantly at any point in the system. This state of affairs favors reaction with other molecules, and it may also result in some special

energy-transfer mechanisms. For example, in most species of plants and algae, chlorophyll molecules are always associated closely with carotenoid pigments such as  $\beta$ -carotene (Fig. 1-9) (Rabinowitch, 1945, p. 412). It seems entirely possible that the carotenoid acts as a power line between chlorophyll molecules to increase the efficiency of light use.

### 3. ENERGY TRANSFER DURING ADIABATIC COLLISIONS

#### 3-1. ABSOLUTE THEORY OF COLLISIONS

Most energy-transfer processes take place during the close approach, or collision, of atoms or molecules. When these processes do not involve a change in electronic quantum numbers, they are termed "adiabatic" after Ehrenfest (1916). It has been customary to treat collisions as the impacts of balls, the properties of the balls being refined as the theories gain sophistication. There is, however, considerable merit in a discussion based on the movement of a configuration point on potential-energy surfaces, as outlined in the preceding section. Some examples of the application of this approach have been summarized by Glasstone *et al.*, (1941, pp. 103-107). Eyring *et al.* (1935) were the first to develop the idea quantitatively, and it has been extended by Gershinowitz (1937) and by Hulburt and his associates (1943, 1950, 1951). Fully quantitative treatments have not been numerous because of the complexity. As a method for understanding, the use of potential-energy surfaces is unexcelled, and we propose to extend in brief formalism the theory of absolute reaction rates to collision processes. No calculational utility is lost in this treatment, since at ultimate simplification the absolute theory reduces to simple collision theory (Eyring, 1935).

Collisions in which no vibrational degrees of freedom change quantum numbers are governed by Newtonian mechanics, since essentially all translational and rotational energies are available. That is, translational and rotational quanta are so small that they allow very nearly a continuous spectrum of energies. Hydrogen and heavy-metal hydrides are exceptions, because they have a very small moment of inertia and hence large rotational quanta. Vibrational quanta, on the other hand, are usually large, so that only a few discrete energy values  $E_v(n)$ , in which  $n$  is the vibrational quantum number, are available for vibrational degrees of freedom. The correspondence limit provided by classical mechanics does not apply, and such degrees of freedom must be treated by quantum mechanics. The simplest example of an energy exchange involving vibrational quanta is the reaction



in which  $A$  is an atom,  $XY$  is a diatomic molecule, and  $A^*$  is a translationally excited atom. If the translational energy before collision is

$E_t$  and after collision is  $E'_t$ ,  $n$  is the vibrational quantum number, and  $\nu$  is the vibrational frequency, the energy-conservation expression for conversion of one vibrational quantum is

$$E'_t - E_t = (n + \frac{1}{2})h\nu - (n - 1 + \frac{1}{2})h\nu,$$

neglecting rotation, as we generally will for purposes of simplified exposition. If now the potential-energy surface for approach along the line of centers, i.e., along the best direction for such a collision, is constructed as in Fig. 1-5, the dotted line, which is the change of configuration with time, represents the collision process. In Fig. 1-10 an enlarged section of a typical collision path is shown. Since vibrational energies must

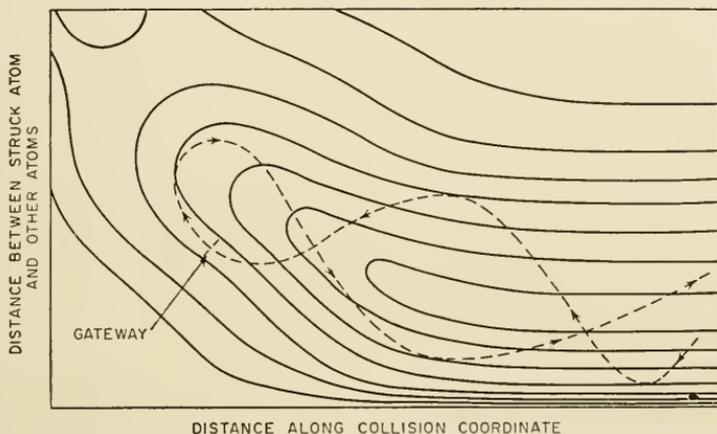


Fig. 1-10. Enlarged drawing of collision trajectory for interconversion of vibrational and translational energy (cf. Fig. 1-5).

change by integral numbers of quanta, the best classical picture is to suppose that all such trajectories of configurations which differ in vibrational energy from the quantized values by small fixed amounts are allowed and that all other trajectories are unsuccessful. The successful trajectories per unit time can then be counted using statistical mechanical expressions in a way analogous to the calculation of reaction rates. The present problem of determining efficiency in energy transfer is somewhat more difficult in that more restrictions are required than in reaction-rate theory. It is therefore necessary to examine several conditions on which the simpler theory rests and extend them to collision processes. We shall not be especially concerned with rotational degrees of freedom, since these may be treated in a complete theory as a special application of what will be said about vibration.

Speaking classically, certain phases of the rotational motion, such as linear collision of molecule and atom or side-by-side approach of two molecules, will be more effective for energy transfer than others. It is generally assumed that the potential surface is calculated only for such

directions of approach. In collision processes the successful trajectories for energy transfer will, in general, cluster together at the place where they reach their highest potential energy. There may be more than one such region or gateway, each with its own highest potential energy  $E_0$ . The rate process will count all successful trajectories just as in chemical reactions. Where more than one saddle point exists for the completion of a chemical reaction, the sum of rates for each saddle yields the over-all rate. In energy-transfer processes, besides the requirement that successful trajectories must fall within the gateway, there will usually be additional conditions that the velocities lie within certain definite limits, whereas for chemical reactions the velocities normal to the barrier may have any value between zero and infinity. In spite of this, we may write for the specific rate constant  $\mathbf{k}$  of any process that can be specified as passage through a gateway the equation

$$\mathbf{k} = \frac{tkT}{h} \frac{f_{\ddagger}^{\ddagger}}{f} e^{-E_0/RT}, \quad (1-11)$$

where  $t$  = transmission coefficient that measures any special properties of the potential barrier which might restrict the passage of a configurational point;

$k$  = Boltzmann's constant;

$T$  = absolute temperature;

$h$  = Planck's constant;

$f$  = single-molecule partition function for the colliding partners in their average states;

$f_{\ddagger}^{\ddagger}$  = partition function of the collision complex at the gateway; and

$E_0$  = highest potential energy per mole reached for the successful trajectory most economical of energy.

The expression  $f_{\ddagger}^{\ddagger} e^{-E_0/RT}$  is the partition function for all trajectories passing through the gateway reduced to an energy zero, which is the zero-point energy of the reactants. Multiplying through by the fraction  $t$ , the transmission coefficient, restricts the trajectories to the successful ones. Clearly such a formalism is necessarily correct, since  $t$  is expressly defined to give the correct value. However, in each particular case the procedure for a priori calculations will be to construct enough trajectories, successful and otherwise, to define the gateway or gateways and then to use quantum mechanics to calculate  $t$  for the established values of the collision energy  $E_0$ . For purposes of discussion and systematization of data, it is convenient to replace Eq. (1-11) by the two-parameter equation

$$\mathbf{k} = \frac{kT}{h} e^{\Delta S_{\ddagger}^{\ddagger}/R} e^{-\Delta H_{\ddagger}^{\ddagger}/RT}, \quad (1-12)$$

where  $\Delta S_{\ddagger}^{\ddagger}$  and  $\Delta H_{\ddagger}^{\ddagger}$  are the apparent entropy and heat for the process in question. If only a small fraction of paths through a gateway are suc-

cessful, the apparent entropy will, of course, be large and negative. A wide gateway means that a number of variations in the position and velocity of the collision paths in the region of the gateway are allowed, and hence less negative entropies of collision will be found. The height of the gateway determines the heat of collision.

For the time being we forgo more elaborate discussion. The matter for emphasis is that simple collision theory is inadequate for energy transfer, just as it is inadequate for rates of reaction. In the new theory, gateways take the place of the saddle points of absolute-reaction theory, and collision processes are interpreted in terms of rate constants that are further expressible as functions of two parameters: one, the entropy of collision, which is not associated with temperature, and the other, the heat of collision, which is.

It has been the usual convention to calculate effective cross sections for the comparison of various energy-transfer processes. These are defined on the basis that every collision results in transfer. For instance, if one knows that the fluorescence of  $N$  excited molecules of a total  $N_1^*$  of type 1 is destroyed per cubic centimeter per second by quenching collisions with type 2 molecules ( $N_2$  per cubic centimeter),  $N$  is taken equal to  $Z$ , the collision number, given by kinetic theory as

$$Z = 2N_1^*N_2\sigma^2 \left[ \frac{2\pi\mathbf{k}T(m_1 + m_2)}{m_1m_2} \right]^{1/2}, \quad (1-13)$$

where  $m$  is the particle mass. The collision cross section  $\sigma^2$  is determined by this condition. Since the collision number is also the specific rate constant for quenching, the relation between the rate constant  $\mathbf{k}$  and  $\sigma^2$  is also expressed by Eq. (1-13).

### 3-2. ENERGY TRANSFER INVOLVING ROTATIONAL DEGREES OF FREEDOM

As depicted in Fig. 1-10, we may discuss gateways of maximum restriction for any collision path. One or a few of these gateways are probably of primary importance in any particular collision process. Furthermore there is no reason that the relative importance of different gateways cannot change with temperature, just as different saddle points can vary in importance for reactions in different temperature regions. The data on the thermal dependence of energy-transfer processes are insufficient for a general picture to be formed, but we do know quite generally that increased temperature causes increased efficiency in transfer. The amount of change for a given temperature increment varies considerably. It is smallest for processes involving only translational and rotational energy, since the heats of collision are small. Translational processes are essentially trivial, though the determination of an effective interaction potential in any case is difficult. Rotational processes, as

previously emphasized, can be represented by figures of the type of Fig. 1-10, though the potential-energy surfaces would have to be calculated for a different "best" collision configuration. Such surfaces would be noteworthy only in that they lie generally at a lower potential energy than surfaces appropriate for vibrational collision. Important gateways would be found to have lower collision energies or heats, since the amount of mutual perturbation of the electrical fields of the collisional participants necessary for the transfer of the small rotational quanta is small, and close approach is unnecessary. The gateways will, because of their position, also probably be rather wide. Both entropy and heats of collision will consequently be such as to favor rapid energy exchange and hence rapid thermal equilibration among rotational and translational degrees of freedom. Anomalous persistence of rotation has been observed only in molecules containing hydrogen:  $H_2$  (Smith, 1936) and  $HgH$  (Rieke, 1936, 1937; Beutler and Rabinowitch, 1930; Oldenberg, 1931; Wood and Gaviola, 1928; Gaviola and Wood, 1928). The behavior of hydrogen is, as we have previously mentioned, due to the large rotational quanta, which require gateways for collision at high potential energies and thus make for poor energy exchange with translational degrees of freedom. In transient phenomena of very high speed, there may be inadequate time for the 1-100 collisions required to achieve normal rotation-translational equilibrium. It has been indicated that the rotational degrees of freedom of some molecules are inert in shock-wave propagation (Lewis and Van Elbe, 1939; Greene *et al.*, 1951). Landau and Teller (1936) have treated equilibration of rotational degrees of freedom. They neglect chemical affinity of the reacting partners. Their treatment is a good first approximation because the collision complexes are easily formed in low-energy reactions of this type.

### 3-3. ADIABATIC PROCESSES OF VIBRATIONAL-ENERGY EXCHANGE

Low-lying equipotential lines are seldom greatly distorted in contour maps for collision processes similar to those shown in Fig. 1-10. This situation is the result of negligible chemical interaction of the collision partners at distances larger than the order of a kinetic-theory diameter. Collision processes that have their principal gateways in the regions of these lines do not depend on chemical affinity. For instance, rare gases should be as good as highly reactive halogen atoms in establishing rotational equilibrium. On the other hand, exchange collisions involving vibrational degrees of freedom, with the exception of heavy molecules like iodine (Roessler, 1935), generally have their important gateways high on the potential barrier, i.e., high on the barrier that restricts chemical reaction. The latter is, of course, the same barrier that limits collision, for, according to the present picture, a collision is no more than an incomplete chemical reaction. The potential height of the gateway

and its availability will largely be functions of the chemical affinity of the collision partners. With the exception of ions of like sign, all pairs of molecules will have at least some positive minimum of mutual affinity. When this minimum is very low, as, for example, it is when one collision participant is a rare-gas atom, distortion of the equipotential lines will occur only at very close distances of approach and hence at high values of potential energy. The lower-lying equipotential lines will be symmetrical parabolas with parallel asymptotes. These parabolas tend to reflect the trajectory without change in vibrational quantum number.

TABLE 1-1. EFFECT OF VARIOUS GASES ON DISPERSION OF SOUND IN OTHER GASES

Added gas	No. of collisions required to dissipate 1 quantum of vibration at 20°C (measured in gas phase)		
	O <sub>2</sub>	CO <sub>2</sub>	Cl <sub>2</sub>
None	500,000	47,000	34,000
N <sub>2</sub>	100,000	.....	43,000
A	.....	47,000	32,000
He	.....	1,700	900
H <sub>2</sub>	20,000	480	780
CO	.....	.....	230
CH <sub>4</sub>	.....	2,400	190
HCl	.....	130	120
H <sub>2</sub> O	400	40	
CO <sub>2</sub>	25,000		
H <sub>2</sub> S	4,200		
C <sub>2</sub> H <sub>5</sub> OH	120		
References	Kneser and Knudsen, 1935	Eucken and Becker, 1934; Eucken and Jaacks, 1935	Eucken and Becker, 1934; Eucken and Jaacks, 1935

The rare gases are consequently poor agents for the establishment of thermal equilibrium in internal degrees of freedom. Helium is superior to the others in this respect, as determined by its effectiveness in preventing the dispersion of sound (Table 1-1). This behavior may be attributed to the small size of the atom which favors close approach and high relative translational velocities. High velocities drive the trajectories onto the higher regions of the potential surface in which the gateways are located.

Studies of the dispersion of sound have provided a major part of the information on collisional exchange of low-energy quanta. Sound in liquid or gas phases is transported in compression waves, which produce alternate high and low pressures in a given small region. Since the process is essentially adiabatic in a thermodynamic sense, the temperature

must follow a similar cycle, and with it the Boltzmann distribution of energies among the various degrees of freedom. At high supersonic frequencies, vibrational-translational interaction will be inadequate to establish thermal equilibrium with internal degrees of freedom, and the heat-capacity (determined from velocity  $v$ ) measurements by means of the well-known formula

$$v = \left( \frac{C_P}{C_V} \cdot \frac{P}{\rho} \right)^{1/2} \quad (1-14)$$

(where  $C_P$  and  $C_V$  = heat capacity at constant pressure and volume, respectively;  $P$  = pressure; and  $\rho$  = density) will not contain the contribution from internal modes of motion. In the neighborhood of some critical frequency dependent on the material, the physical state, and the temperature, energy will be restored to external degrees of freedom just  $180^\circ$  out of phase with the compression wave. As a result, the wave will be damped and retarded, thus producing the phenomena of absorption and dispersion of sound. Sound dispersion has been thoroughly treated both experimentally and theoretically (reviewed by Richards, 1939). Some important theoretical treatments are due to Kneser (1931, 1933), Herzfeld and Rice (1928), Saxton (1938), and their various coworkers. The energies involved are frequently small with respect to those of the reaction potential barrier, so that chemical effects may not be important. That is, the collision paths generally lie about the lower equipotential lines, which are seldom distorted. It is to be expected that the persistence of vibrational energy will consequently be considerable. Nevertheless, as Eucken and Becker (1934) and Eucken (1935) pointed out for sound dispersion, chemical affinity is the most important consideration in these processes. Table 1-1 illustrates the importance of affinity, which can probably best be explained as due to a general lowering of the surfaces rather than to an increase in distortion. Like molecules have considerable affinity for each other, but it is clear that trace substances with high dipole moments or high reactivity, such as water and carbon dioxide, for instance, are frequently even more effective in preventing sound dispersion.

The number of collisions required for equilibrium can be most readily calculated after the manner of Bethe and Teller (1940). Diatomic molecules treated as harmonic oscillators are considered, since multiply vibrating molecules add little save complexity. The circular acoustic frequency of maximum absorption of sound per wave length  $\omega_{\max}$  can be determined from experiment. It is related to the relaxation time  $1/\omega_0$  for the equilibration of internal degrees of freedom by the expression

$$\frac{\omega_0}{\omega_{\max}} = \left( \frac{C'_P[C'_P - R]}{C_P[C_P - R]} \right), \quad (1-15)$$

in which  $C_P$  is the total specific heat and  $C'_P$  is that for the external degrees of freedom only. Now, if  $\nu$  is the vibrational frequency of the molecules,

$$\omega_0 = \mathbf{k}_{10}(1 - e^{-h\nu/kT}), \quad (1-16)$$

in which  $\mathbf{k}_{10}$  is the rate constant for the deexcitation from the upper vibrational state to the lowest state and is related to the reverse constant  $\mathbf{k}_{01}$  by the principle of detailed balancing thus:

$$\mathbf{k}_{01} = \mathbf{k}_{10}e^{-h\nu/kT}, \quad (1-17)$$

the rate constant in terms of experimental quantities is

$$\mathbf{k}_{10} = \omega_{\max} \left( \frac{C'_P[C'_P - R]}{C_P[C_P - R]} \right)^{1/2} / e^{-h\nu/kT}. \quad (1-18)$$

The reciprocal of  $\mathbf{k}_{10}$  divided by the half-period of oscillation gives directly the number of collisions required to transfer a single vibrational quantum. The rate constants are, of course, the sums of all successful collision trajectories regardless of original vibrational phase (classical sense), relative translational motion, and number of participating gateways. From the numbers of collisions calculated in this way, which are listed in Table 1-1, it can be seen that the exchange of energy between vibrational and translational degrees of freedom is a very poor process, especially if one of the partners is chemically inert or the bond involved is strong. The gas  $\text{N}_2$ , which is quite unreactive and has a very high bond energy of the order of 200 kcal/mole, requires more collisions than does  $\text{O}_2$  with a bond energy of 117 kcal. The gas  $\text{O}_2$ , in turn, requires many more collisions than  $\text{Cl}_2$ , which has a bond strength of 58 kcal/mole. Hydrogen is very efficient in energy transfer. It has a decided affinity for many substances, and its small size favors close approach and high translational velocities (Eyring, 1935; Hirschfelder *et al.*, 1936).

#### 3-4. COLLISIONS OF POLYATOMIC MOLECULES

When polyatomic molecules undergo collision, further complexity is introduced into any exact calculation by the fact that any atom will participate in many normal modes of vibration, all of which may have to be treated in the calculation. The essential process is still the interaction of two atoms, one in each molecule. The problem has therefore been treated as the collision of atoms with additional factors for the coupling to various degrees of freedom of these atoms. Two semiclassical treatments employing simple repulsive potential functions are due to Jackson and Mott (1932) and to Zener (1931). Zener's treatment is particularly simple and provides a model for promising developments currently in progress. He used the potential function

$$Ce^{-r/d}, \quad (1-19)$$

in which  $C$  and  $d$  are empirical constants,  $d$  being a characteristic bond radius of about 0.35 Å (Zener, 1933b), and  $r$  is the distance between centers of gravity of the colliding atoms in the collision complex. The probability that a given inelastic collision will occur can be expressed as the product of factors,

$$p = p_0 p_1 p_2 \cdots p_i \cdots p_n, \quad (1-20)$$

in which  $p_0$  includes translational aspects of the collision. It is a function of  $\epsilon$ , the energy that must be transferred between translational degrees and vibrational degrees of freedom, and  $\mu$ , the reduced mass of the colliding systems. The term  $p_0$  increases rapidly with  $\mu$  and decreases more rapidly with increasing  $\epsilon$ . The function  $p_0$  has been tabulated for a number of values of the variables. The factors  $p_i$  measure the probability that the  $i$ th degree of freedom will undergo a necessary change in quantum number. There will be one such factor for each of the  $n$  internal degrees of freedom involved in the transfer. The  $p_i$ 's are approximated by

$$p_i = \left( \frac{\beta_i s_i}{d} \right)^2, \quad (1-21)$$

in which  $\beta_i$  measures the coupling of the struck atom with the  $i$ th vibrational coordinate; i.e., it is the coefficient of that particular coordinate when the displacement of the struck atom is expanded in terms of the normal coordinates. The term  $s_i$  is the matrix element of the coordinate of the  $i$ th normal mode between ground and the particular excited state  $v$  involved. It may be calculated in any particular case by the method of Sommerfeld (1932). Applying the procedure to deexcitation of  $O_2$  from its first vibrational excited state, Zener (1935) found  $p_0 = 10^{-5}$  for the  $\epsilon$  of 5.3 kcal/mole,  $p_1 = 6 \times 10^{-4}$ , and thus  $p = p_0 p_1 = 6 \times 10^{-9}$ . The rate constant for the process is the product of this probability times the kinetic-collision frequency. The calculation is too small and can be improved by choice of a better potential function, for example, that of Jackson and Mott. The theory is, of course, very approximate but gives fair relative agreement with the measurements of sound dispersion and the transfer of thermal energy both in the bulk phase and from surfaces to gases. The calculation of accommodation coefficients measuring the efficiency of the latter processes has been treated by Jackson and associates (1932, 1933, 1935).

An absolute-rate theory for collisions of polyatomic molecules along the lines outlined in this chapter has little practical importance because of the extreme complication of the potential surfaces for such molecules. Similarly the Born type of scattering calculation for molecular beams (Massey and Burhop, 1952) is extremely difficult to apply even to small molecules. A promising attack on the problem has recently been started along the lines of the Zener theory, i.e., isolation of the different types

of coordinates, which can then be treated independently (Curtiss and associates, 1950, 1952).

Returning to Zener's work, we find that his calculation for the efficiency of transfer of the large vibrational quantum of the nitrogen molecule from one molecule to another gives the high value of  $10^{-5}$ , which is compatible with observation. This result is due to the participation of just two internal degrees of freedom, in turn the result of the exact balance of energy available against energy that can be taken up by the second molecule. This is the condition for maximum efficiency and is called the "resonance condition" or "resonance requirement." Its explanation is especially clear in Zener's theory: the fewer  $p_1$ 's involved, the better; the less the energy that must be added or taken from a third degree of freedom, the greater the probability of transfer. Franck first proposed this explanation.

Qualitatively we can understand that nitrogen should be efficient in any transfer process despite its normally low chemical reactivity. The more strongly a bond oscillates, the more the atoms take on the properties of free atoms and so tend to combine more strongly with other atoms that come within range. The result is that a rapidly vibrating oscillator, when it strikes a second oscillator, will cause the second one to expand and absorb energy. This makes for rapid communication of energy between high-frequency oscillators, however they collide. Molecules like water can be very efficient in transfer reactions despite their lower vibration frequencies because they possess chemical affinity for many substances. Thus nitrogen causes the collision path to climb high on a high-potential-energy barrier, whereas water reduces the height of the barrier, making extreme interpenetration of the colliding partners unnecessary. In addition, the vibrational quanta of water are intermediate in energy between the high-energy quanta of oxygen or nitrogen and the small quanta of translation and rotation.

Further information about energy-transfer processes in collisions on single potential-energy surfaces has been secured from the effectiveness of substances as third bodies in triple collisions leading to simple atomic combination. A third body is necessary in such cases to remove the extra energy so as to stabilize molecule formation. The rates of unimolecular reactions, the transfer of heat, and the study of shock-wave propagation have also provided information.

Energies of the order of 100 kcal/mole are produced in atomic combination, but only a fraction of this need be removed by a third body to stabilize the formation of diatomic molecules. Originally this energy is stored as high vibrational amplitude in the collision complex. Such amplitudes favor penetration of higher potential surfaces with a consequent increase in the probability of finding a suitable gateway. It has been well established that chemical affinity is of prime importance in

these processes (Kassel, 1932, p. 313; Eyring, 1935; Boehm and Bonhoeffer, 1926; Rabinowitch and Wood, 1936a,b; Hilferding and Steiner, 1935). In terms of the requirement for matching of quantum magnitudes in energy exchanges, the observations on stabilizing collisions can be correlated with the fact that the size of vibrational quanta decreases with increasing quantum number. The higher this number, the better the probability for transfer into translational energy.

Third bodies that are effective in stabilizing recombination are, of course, equally efficient in providing activation energy for unimolecular reactions. Excited molecules have a much higher probability of losing energy on the next collision than of gaining additional amounts (Roessler, 1935). Molecules with few internal degrees of freedom are consequently activated from the median-energy population. The upper regions of potential energy are involved in activating collisions, and hence, as observed, chemical affinity plays a very important role (Glasstone *et al.*, 1941, Chap. 5).

*Intramolecular Energy Migration.* Molecules with many internal degrees of freedom may store energy preceding a final unimolecular reaction step. Requirements for activating collisions will then be less strict, since the process can occur through several lower-energy collisions.

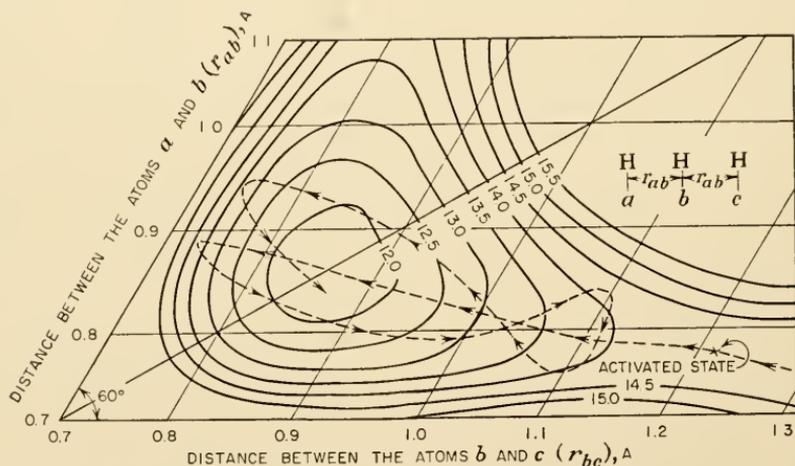


FIG. 1-11. Calculated trajectory for migration of vibration in the hypothetical molecule  $H_3$ . (From Hirschfelder *et al.*, 1936.)

That is, the slow process may shift from transfer to the molecule to transfer within the molecule. Internal migration of energy is extremely complicated and has not yet been dealt with satisfactorily. The representation of such processes involves trajectories that cross and recross the stable potential wells found on such surfaces as that of Fig. 1-4. The appropriate well for any molecule will have as many dimensions and as many valleys as there are independent vibrations. In Fig. 1-11

the first several oscillations are shown as they were calculated by Hirschfelder *et al.* (1936). The penetration of each valley by the collision path is similar to the collision between two diatomic molecules and could perhaps be treated as an independent collision process involving small parts of the molecule treated as isolated entities. The fact that most of the atoms of a molecule are participants in several independent modes of motion greatly complicates such a calculation. This condition also improves the probability of energy migration. Using the lowest point of the potential well as the origin, the potential energy may be expanded classically in a Taylor's series thus:

$$E = \sum_{i=1}^n \left( \frac{\partial V}{\partial q_i} \right)_0 q_i + \frac{1}{2i} \sum_{i=1}^n \sum_{j=1}^n \left( \frac{\partial^2 V}{\partial q_i \partial q_j} \right)_0 q_i q_j + \frac{1}{3!} \sum_{i=1}^n \sum_{j=1}^n \left( \frac{\partial^3 V}{\partial^2 q_i \partial q_j} \right)_0 q_i^2 q_j + \dots \quad (1-22)$$

Since the first-order terms are zero and choice of the  $q$ 's as normal coordinates eliminates the second-order cross terms, the energy, which is not strongly dependent on the third-order terms, may be expressed as a sum of independent second-order terms. However, energy migration is entirely due to the third-order cross terms, which do not vanish. In Fig. 1-12 idealized potential wells for the cases with and without these so-called "coupling" terms are pictured. Trajectories in the smooth well preserve energy in its original distribution. The distortions of the second well are necessary for migration.

The third-order coupling coefficients are sufficiently large to allow reasonably free exchange of energy among all degrees of freedom, as Franck *et al.* (1932) emphasized. The rapidity of such energy migration may be explained by the shallowness of potential wells for stable molecules. The collision path always moves in the region of suitable gateways. The density of these

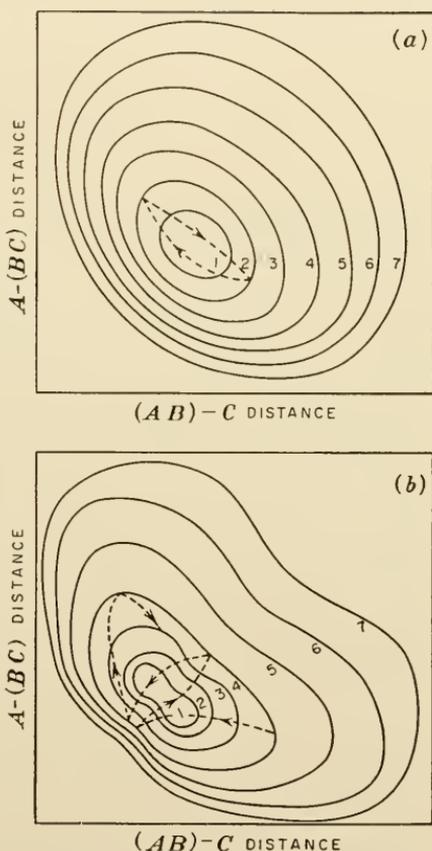


FIG. 1-12. Idealized potential wells for stable diatomic molecules: (a) without coupling term between modes and (b) with coupling term.

gateways increases as the number of vibrations. When there are many gateways, perhaps every vibration of each mode results in some energy transfer. Teller (1941) has described the quantum-mechanical mechanism in the following way: Certain wave functions of each mode will correspond in energy eigenvalues to certain wave functions of other normal modes. When pairs of wave functions correspond to the same energy, they recombine to form a new independent pair, each mode of which contains elements of the original pair. The energy is redistributed in the time allowed before the degeneracy is destroyed by further coupling with other modes.

In terms of absolute collision theory, it can be seen that the rate constants for energy transfer in internal collisions may be of considerable magnitude. The rate constant after cancellation of single-coordinate partition functions, which are identical for the average and activated forms of the molecule, looks like this:

$$\mathbf{k} = t \frac{kT}{h} \frac{1}{1 - e^{-h\nu_1/kT}} \frac{e^{-E_0/RT}}{1 - e^{-h\nu_2/kT}} \quad (1-23)$$

One vibrational partition function of the average molecule approximately cancels the single vibrational partition function of the activated molecule, and since the remaining term in the denominator is nearly unity, the rate constant will take on a value limited only by the collision energy, which is generally low, and the transmission coefficient, which can be relatively large because of the types of potential surfaces involved. The average time for this exchange will vary from mode to mode and molecule to molecule, but it is undoubtedly no faster than a fast vibrational time, i.e., about  $10^{-14}$  sec, and this only when the molecule is large and the collision energy nearly zero. Arrhenius frequency factors for these processes, or indeed for any processes that depend on the motions of nuclei, must therefore seldom exceed  $10^{13}$ , though some increase can be expected as a result of coincident transfers taking place at other parts of the molecule.

Lack of precise knowledge of an average time for a single transfer seriously limits the applicability of the Kassel and Rice theory for unimolecular reactions, though this is the only widely used theory for these reactions. Even for small molecules the details of quantum size and the strength of coupling between modes are rarely known, so that the theory of Kassel (1932, p. 93) and Rice assumes an Einstein-like single vibrational frequency applicable to all modes of motion. The procedure is to calculate the probability  $p$  that  $m$  of a total of  $j$  uniform quanta will be found in a given one of  $s$  vibrational modes. Two forms of this expres-

sion are

$$p = \frac{j!(j - m + s - 1)!}{(j - m)!(j + s - 1)!} \quad (1-24)$$

and

$$p = \left(\frac{j - m}{j}\right)^{s-1}, \quad (1-25)$$

the latter applying at large  $j$ , i. e., at classical conditions. Reaction occurs when  $m$  or more quanta become localized in the bond. The probability, summed for all  $m$  values greater than some critical value, multiplied by the reciprocal of the average time for single-quantum transfers gives the rate constant under reaction conditions such that the velocity is limited by internal transfers of energy. The theory is not especially satisfactory in either form because of the poor knowledge of the average time for any large molecule. Similar limitations apply to the calculation of the rate of dissipation of photoenergy absorbed and converted to vibrational energy. In small molecules this energy may be used efficiently because there are few degrees of freedom into which it may be dissipated. In large molecules the energy will be greatly diluted in the many degrees of freedom, so that quantum yields for photoreactions can be expected to be low. For instance, photodenaturation of proteins is usually an inefficient process with low quantum yields (McLaren and Pearson, 1949; Katchman and McLaren, 1948; Kubowitz and Haas, 1933).

A number of attempts have been made toward improving the Kassel-Rice theory (Marcus, 1952; Slater, 1948; Benson, 1952). The problem is very complicated in molecules even of small size because energy migration involves all atomic distances. Simplified potential surfaces are completely inadequate for the problem. Apart from a knowledge of the complete surfaces, the absolute-rate theory for unimolecular processes is reasonably complete (Glasstone *et al.*, 1941, Chap. 5; Giddings and Eyring, 1954; Magee, 1952). Giddings and coworkers have observed that negative entropies of activation for unimolecular reactions are impossible, and their apparent occurrence in unimolecular reactions must be explained by small values of the transmission coefficient. They reason that the reverse process, i.e., bimolecular combination of radicals, is a poor process in that the activated complexes are reflected back to reactants through their inability to dissipate energy sufficiently rapidly among internal degrees of freedom. Since the transmission coefficient must be the same independent of the direction of the reaction, it will also be small for unimolecular decomposition. That is, the process by which energy previously stockpiled in internal degrees of freedom is delivered to the reacting bond is also poor. The ability of the internal degrees to dilute the extra energy of the molecule varies with the amount

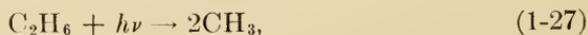
of this energy. Thus the transmission coefficient will vary with the state of the activated complex, and not all activated complexes will be alike. This behavior is in contrast to that observed in other types of reactions. The problem is further complicated by the fact that at any instant most of the activated complexes in any given energy state will be those just returning from an unsuccessful attempt to pass the barrier. This situation destroys equilibrium distribution of complexes moving toward the barrier and makes necessary further corrections depending on the transmission coefficient for the state involved. It is just these transmission coefficients that depend on internal coupling and the full potential-energy diagram for the molecules.

Activation energies are not usually observed in photoreactions because the excitation energy exceeds the thermal requirement for the reactions that occur. The thermal values of the activation energy provide a lower limit for the photoenergy required if thermal and photoreaction mechanisms are identical. Thermal energies can occasionally make up a deficit in the energy for a photoreaction (Franck and Herzfeld, 1937). An interesting example is the discovery by St. George and Wald (1949) that mammalian vision has a temperature coefficient at the red end of the spectrum in contrast to the absence of a thermal dependence at shorter wave lengths. The observation can be explained readily as a deficiency of long-wave-length radiation for the primary process of vision. The thermal increment may serve as activation energy for crossing to a lower potential surface or as activation energy for the subsequent process on the lower surface. In the photolysis of  $I_2$  (Rabinowitch and Wood, 1936b) and  $S_2$  (Durand, 1940), collisional transfers of energy provide the necessary extra energy for these molecules, when photoexcited, to reach their crossing points.

Emphasis must be placed on the activation rather than the thermodynamic energy requirements of a given photoreaction. In diatomic molecules the only allowed unimolecular process is bond rupture, in which the heat of reaction and the activation energy are identical. In larger molecules there are usually alternative chemical processes that can occur with lower activation energies. Consider the case of ethane photolysis in the gas phase studied at 1470 and 1295 Å by Faltings (1939). The quantum yields  $\Phi$  are  $\Phi_{H_2} = 0.96$ ,  $\Phi_{C_2H_2} = 0.20$ ,  $\Phi_{C_2H_4} = 0.56$ ,  $\Phi_{CH_4} = 0.05$ , and  $\Phi_{C_3H_6+C_4H_{10}} = 0.04$ , indicating preference for the primary reaction



with the low activation energy  $E_0$  of about 20 kcal/mole. Some methyl radicals are also produced by the reaction



with  $E_0 = 78$  kcal/mole. The difference in yields is explained by the

considerable difference in activation energies. These and similar processes substantiate the previously discussed belief that vibrational energy migrates with ease throughout molecules. There is no apparent requirement based on geometry except perhaps in large molecules, in which low-energy-reaction regions are isolated from chromophoric groups by inadequate coupling through weak or too few bonds.

There are a few general rules for efficient energy transfer in adiabatic collision processes, owing, as we have seen, to the properties of the potential-energy surfaces involved:

1. Chemical affinity favors energy transfer. An upper limit of affinity is clearly compound formation between the participants, in which case the migration of vibrational quanta goes on under the most efficient conditions.

2. High relative velocity of the atoms primarily concerned in a given collision process is generally favorable to collisional transfers, since activation (collision) energies are required. In contrast to the situation in chemical reactions, energies in excess of precise values may reduce rather than increase the rate of migration.

3. Energy transfer is most probable when the fewest degrees of freedom change quantum numbers.

#### 4. ENERGY EXCHANGE IN DIABATIC PROCESSES

##### 4-1. TYPES OF DIABATIC PROCESSES

By analogy with the term "adiabatic," "diabatic" processes are defined as those which occur with a change in electronic quantum number. For convenience in treating photoinduced processes, we divide these into two classes. In the first class are included those reactions of molecules which occur unimolecularly after the absorption of radiation. Excitation is to an unstable electronic state or to a stable state from which internal conversion takes place rapidly and spontaneously. In the latter instances the vibrational energy thus produced is dissipated in unimolecular reaction or as heat during the collisions that follow. For convenience we also include those cases in which internal conversion is induced by perturbations of other molecules that do not profit by energy gain or reaction from the process. For instance, the effectiveness of ions as quenchers is frequently attributed to their perturbing reaction (see Sect. 2-1). The migrations of vibrational energy in the excited state preceding internal conversion, as well as the fate of vibrational energy following the crossing of potential surfaces, can be treated at best only very approximately by the methods of Sect. 2. The problem is further complicated on upper surfaces by the lack of knowledge of the position of crossing points.

Molecules of the first-class reactions seldom fluoresce, so that internal conversion usually occurs in less than  $10^{-8}$  sec. Most small molecules are of this type when irradiated with wave lengths of energy greater than bond energies. The absorption spectrum for such wave lengths is a continuum. At longer wave lengths a diffuse rotational fine structure is frequently observed, though the vibrational lines are sharp. These diffuse spectra, termed "predissociation" spectra, give some estimates of the times required for the crossing conditions to be met in upper surfaces. The excited molecule remains on the upper surface long enough to allow development of the vibrational structure but crosses before rotation becomes fully realized. The calculation of the time before crossing requires the use of the Heisenberg uncertainty principle after the manner of Bonhoeffer and Harteck (1933). The form of the principle useful here is

$$\Delta\nu \cdot \Delta t = 1, \quad (1-28)$$

in which  $\Delta\nu$  is the uncertainty in frequency and  $\Delta t$  is the uncertainty in lifetime. Rotational lines are on the average 100 cal apart, corresponding to a frequency of  $10^{11}$  sec<sup>-1</sup>. To produce the observed diffuse band structure,  $\Delta\nu$  must be of the same order as the separation of lines in a sharp spectrum. Hence

$$\Delta t = 1/\Delta\nu = 10^{-11} \text{ sec}, \quad (1-29)$$

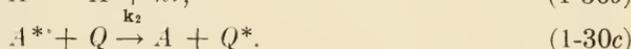
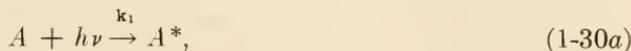
where  $\Delta t$  is now the average lifetime in the excited vibrational state. It varies from case to case, but this value may be taken as a suitable average (Rice, 1933; Burton and Rollefson, 1938; Noyes and Henriques, 1939; Rosen, 1933; Kimball, 1937). The lifetime is long with respect to the interval between collisions with solvent molecules which will remove the extra energy. It is short with respect to the time between collisions with other solute reactants. Hence we see that a second molecule different from the solvent must be very near the excited molecule at the time of excitation to receive any of the energy. Such a molecule must further compete with the very rapid internal processes of the excited molecule and will generally be the loser.

In the second class are included those reactions in which crossing is induced by a quencher in a molecule which would otherwise fluoresce and in which the quencher receives some portion of the extra energy. These reactions, termed "sensitizations," form a major part of the domain of photoreactions. The second class may be further divided into two subclasses: those reactions in which the quencher receives its energy in vibrational, rotational, and translational forms; and those in which the quencher undergoes electronic excitation. Only infrequently for small molecules are the two classes likely to overlap in the sense that vibrational or translational energy is reconverted in the quencher to electronic energy.

Diabatic sensitization reactions are the primary concern of this section. Most information on such reactions has come from studies of the quenching of fluorescence.

#### 4.2. THE QUENCHING OF FLUORESCENCE

Most quenching reactions follow some variant of a mechanism originally suggested by Stern and Volmer (1919):



The efficiency of substances as quenchers is measured by the rate constant  $k_2$ , which is related to the light absorbed per second  $I_a$  and the fluorescence intensity  $I_f$  by

$$k_2 = k_{-1} \cdot \frac{1}{[Q]} \left( \frac{I_a}{I_f} - 1 \right), \quad (1-31)$$

so that  $k_2$  values can be calculated once  $k_{-1}$  is determined in other experiments. More detailed discussions of quenching mechanisms can be found in Rollefson and Stoughton (1941) and Rollefson and Boaz (1948). For a majority of cases exact kinetic schemes have not been established.

We may distinguish three types of quenching reactions, and hence three interpretations of  $k_2$ . In the first, every collision between excited and quenching entities immediately destroys quenching entities. Diffusion is rate-limiting. The value of  $k_2$  will vary with the viscosity of the solution but not with the chemical properties of the quencher. When the primary molecule is large with respect to the quencher, the rate constant may be expressed approximately in terms of an expression due to Smoluchowski (1918) for the coagulation of particles,

$$k_2 = 4\pi DR, \quad (1-32)$$

in which  $R$  is the effective quenching radius of the excited molecules and  $D$  is the diffusion constant, which can also be written as

$$D = \lambda^2 t \frac{kT}{h} e^{-\Delta F^\ddagger/RT}. \quad (1-33)$$

In this expression  $\lambda$  is the length of individual jumps of the quenching molecules through solution and is equivalent to the mean free path in a gas-phase reaction. The other symbols have their usual meanings (Glasstone *et al.*, 1941, p. 477). It is ordinarily necessary to employ a time-dependent expression for the amount  $A$  reacted in time  $\tau$  at constant incident light intensity:

$$A = 4\pi RDc_0 \left[ 1 + \frac{R}{(\pi D\tau)} \right], \quad (1-34)$$

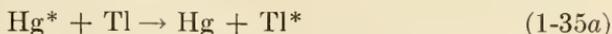
in which  $c_0$  is the original concentration of quenching agent. This equation, also due to Smoluchowski (1918), is a fair first approximation but fails, as does the Stern-Volmer mechanism, at high  $D$  and high  $c_0$ . Corrections for these and electrostatic effects have been given by Montroll (1946), Umberger and LaMer (1945), and Grand *et al.* (1951), so that it is now possible to relate rate constants for quenching to those for diffusion in a variety of cases. Some examples of diffusion-limited reactions thus treated are the quenching of uranin fluorescence by aniline and of riboflavin fluorescence by potassium iodide (Grand *et al.*, 1951). The effective quenching radii determined in this way are usually less than the combined kinetic-theory radii of the participants. Collision cross sections calculated from simple collision theory, on the other hand, are frequently much larger (Baxter, 1930; Boechner, 1930). The mean lifetimes can be determined with high precision and are satisfactorily in agreement with lifetimes determined by depolarization of fluorescence. Fluorescence produced by an initially polarized incident light beam is polarized if the viscosity of the solvent hinders rotation of the excited molecule until emission can take place (see, for instance, Perrin, 1926; Pringsheim and Wavilow, 1926; Lewschin, 1924). A study of depolarization versus viscosity yields the mean lifetime of excitation.

Diffusion-limited processes afford scant information on energy exchange between molecules. Quenching-rate constants determined when the interaction of participants is rate-limiting are more helpful. In solution reactions, viscosity has little effect on rate, but chemical properties do affect it. Quenching reactions of this type alone are of interest in this discussion of energy transfer. However, we must include for completeness a third interpretation of the quenching constant  $k_2$ , different from the first two discussed and of minor significance since such cases occur rarely if at all. In this last case the quencher enters and escapes from the vicinity of the excited molecule several times during the lifetime of excitation. The constant  $k_2$  is a conventional bimolecular rate constant and can be expressed in the usual form, given by Eq. (1-12). The reaction, which is independent of viscosity, proceeds like any reaction with a positive free energy of activation.

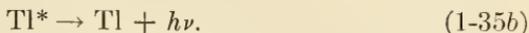
#### 4-3. ENERGY TRANSFER BETWEEN ELECTRONIC DEGREES OF FREEDOM

The simplest example of a process in which electronic excitation energy is transferred directly as such to cause electronic excitation of the quenching molecule is the collision of two atoms in the gas phase. Mercury atoms excited at 2537.5 Å to the  $6^3P_1^0$  state fluoresce with unit quantum yield at low pressures. At higher pressures, collisions of the second kind with other atoms quench the fluorescence. Cario (1922) and Cario and Franck (1923) noticed that thallium atoms were efficient in the quench-

ing reaction, during which their own fluorescence was sensitized thus:



and



Fluorescence occurred from a number of thallium energy levels lying below the energy of the excited state of mercury. It was strongest, however, when the wave length of fluorescence most closely corresponded to the excitation wave length for mercury. From this it was concluded that the transition energy required by the quencher should be closely equal to the excitation energy available in the primary molecule, as it is in mercury and thallium. This requirement appears to be common to many energy-transfer processes and, following the classical definition of resonance, is frequently referred to as the "resonance requirement for energy transfer." Its explanation has been discussed in Sect. 3-4.

Another well-studied example of a quenching reaction of atoms in which the resonance requirement is markedly demonstrated is the quenching of mercury fluorescence by sodium atoms (Beutler and Josephy, 1929). Only those transitions of sodium corresponding to energies available in excited mercury atoms take place in any number. Such processes may be represented in the potential diagrams of Fig. 1-13. A collision between mercury and thallium takes place as the configuration point moves from right to left along the upper curve until the potential-energy barrier reverses the direction of travel and the point moves back out to the right, perhaps on the lower curve. In Fig. 1-13a all collisions will pass the crossing point, so that there is always some probability that the excitation quantum may be transferred. In Fig. 1-13b only those collisions with relative translational energy in excess of a limiting energy  $E_0$ , which we call the collision energy, will pass the crossing point. The rate of cross-

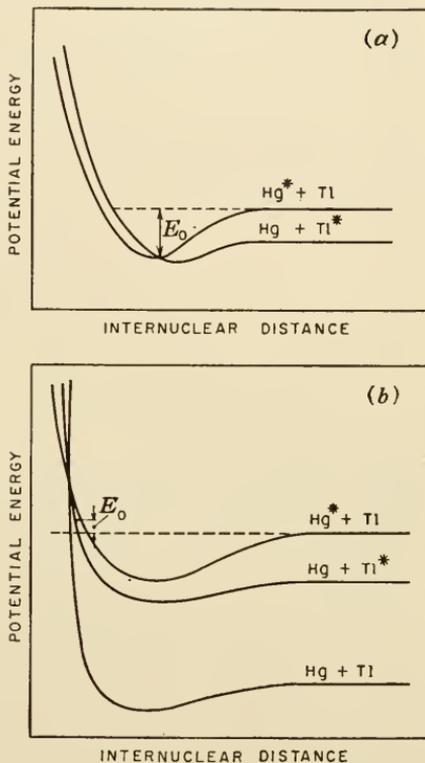


FIG. 1-13. Hypothetical potential surfaces for the transfer of electronic energy from mercury atoms to thallium atoms, demonstrating (a) a negative collision energy and (b) a positive collision energy.

ing and hence of energy transfer can be calculated directly by means of absolute-rate theory:

$$\text{Rate} = [\text{Hg}^*][\text{Tl}]t \frac{kT}{h} \frac{f_{\ddagger}^{\ddagger}}{f_{\text{Hg}^*} f_{\text{Tl}}} e^{-E_0/RT}. \quad (1-36)$$

When all concentrations are expressed in molecules per cubic centimeter, substitution of partition functions converts Eq. (1-36) to

$$\text{Rate} = [\text{Hg}^*][\text{Tl}] \frac{t \frac{kT}{h} \left( \frac{2\pi(m_{\text{Hg}} + m_{\text{Tl}})kT}{h^2} \right)^{3/2} \frac{8\pi^2 kT m_{\text{Hg}} \cdot m_{\text{Tl}} (r_{\text{Hg}} + r_{\text{Tl}})^2 f_{\ddagger}^{\ddagger} \cdot e^{-E_0/RT}}{(m_{\text{Hg}} + m_{\text{Tl}})h^2 \frac{f'_{\text{Hg}} f'_{\text{Tl}}}{f_{\text{Hg}} f_{\text{Tl}}}}}{\left( \frac{2\pi m_{\text{Hg}} kT}{h^2} \right)^{3/2} \left( \frac{2\pi m_{\text{Tl}} kT}{h^2} \right)^{3/2}}, \quad (1-37)$$

in which the primed  $f$ 's are electronic partition functions and the  $r$ 's are atomic radii. It is interesting to note that Eq. (1-37) reduces by cancellation to the simple kinetic collision number  $Z$  times certain correction factors:

$$\text{Rate} = Zt \frac{f_{\ddagger}^{\ddagger}}{f'_{\text{Hg}} f'_{\text{Tl}}} e^{-E_0/RT}, \quad (1-38)$$

where

$$Z = 2[\text{Hg}^*][\text{Tl}] (r_{\text{Hg}} + r_{\text{Tl}})^2 \left( \frac{m_1 + m_2}{m_1 m_2} \right)^{1/2} (2\pi kT)^{1/2}. \quad (1-39)$$

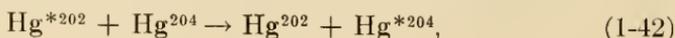
In calculating the value of the transmission coefficient, the crossing-point formula, Eq. (1-9), must be applied twice. The probability for crossing on the right-to-left trajectory is given by  $p$ . On the return passage the chance for remaining in the upper curve is

$$1 - p = 1 - e^{-4\pi^2 \epsilon^2 / h^2 |s_i - s_f|}. \quad (1-40)$$

The order of crossing can also be reversed. Hence, neglecting restrictions on momenta,

$$t = 2p(1 - p). \quad (1-41)$$

Equation (1-41) has its greatest value at  $p = 1/2$ . The crossing formula applies to states of molecules and is not strictly applicable to the crossing between surfaces that describe the energy of two separable chemical entities. It is necessary to introduce another parameter that expresses the interaction potential between the entities as a function of the distance of separation. For efficient crossing there must first exist a crossing point, and, secondly, this point must be at a sufficiently small value of the internuclear distance so that there is an interaction between the partners involved in the exchange process. For instance, in the electronic-energy-transfer process



the potential-energy surfaces for  $\text{Hg}^{*202} + \text{Hg}^{204}$  and  $\text{Hg}^{202} + \text{Hg}^{*204}$  will be completely superimposed, so that crossing points exist at infinite separation of the atoms. The crossing efficiency is limited by the rapid decrease in interaction potential with internuclear or intermolecular distance but may be appreciable over very large distances if the potential surfaces are closely congruent. The formula of Landau and Zener, Eq. (1-9), is clearly not applicable, since  $\epsilon$  and  $|s_i - s_j|$  both go to zero as the surfaces achieve close superposition. A different theoretical approach, initiated in quantum-mechanical form by Kallmann and London (1929), is necessary when the energy discrepancy between initial and final excitation energies becomes small. The observed resonance dependency of transfer efficiency appears in the theories and explains the observations of resonance in thallium fluorescence sensitized by mercury. As perfect resonance is approached, gateways develop at larger internuclear distances and hence at lower potential energies. Similarly the transmission coefficient approaches  $\frac{1}{2}$ , and the rotational partition function increases. All these factors increase the rate of energy transfer, and it can be seen that the dependence of the rate on energy discrepancy can be something like a resonance relation.<sup>7</sup> The relation, approximately as calculated by Kallmann and London (1929), is shown in Fig. 1-14.

As Figs. 1-13*a* and *b* are drawn, the transfer on Fig. 1-13*a* will be more efficient than that on *b* because the collision energy is negative.

It might be expected, by analogy with the former figure, that some quenching processes will demonstrate the unusual phenomenon of negative temperature coefficients. A temperature study of the mercury-thallium quenching reaction could, perhaps, distinguish whether Fig. 1-13*a* or *b* is appropriate.

Fluorescence from the lower electronic levels of thallium, which do not match in their transitions the excitation energy of mercury, may be explained by the occurrence of collisions of the second kind which thallium atoms undergo following their initial excitation to the highest possible level. Little electronic energy need be converted in this way into trans-

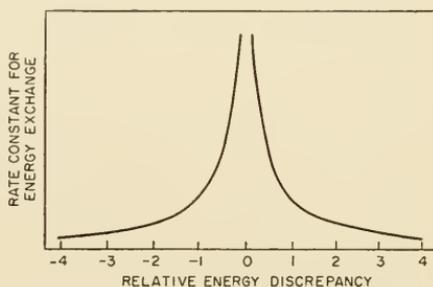


FIG. 1-14. A calculated resonance relation between the rate of energy transfer and the energy discrepancy existing between the excitation energy available in a particular degree of freedom of the primary molecule and the energy that can be taken up by a degree of freedom of a quenching molecule. (After Kallmann and London, 1929.)

<sup>7</sup> That the resonance relation is actually predicted by theory is a matter of considerable complexity. The reader is referred to Mott and Massey (1949), Chaps. 8 and 12, and to Stueckelberg (1932).

lational energy to produce high velocities. Atoms moving with very high velocities are termed "hot" and may in rare instances lose their energy in subsequent collisions to produce excited electronic states. Geometrical and momentum restrictions favor rapid redistribution of the extra energy into smaller translational quanta, and it is well known that electronic and, indeed, vibrational excitation occurs rarely as a result of collisions with hot atoms produced in chemical reactions or by quenching. In stellar atmospheres, conditions such as high relative concentration of hot atoms and long periods between collisions may be such as to make hot atoms important. It is also possible that detonation processes may partly depend on reactions induced by these atoms (Sanger *et al.*, 1949) (Sect. 5). The very hot atoms produced by recoil in nuclear disintegration (Szilard-Chalmers effect) probably cause reaction on their first collision in most cases (Friedman and Libby, 1949). Such atoms are, of course, rare in nature, though it is possible that gene mutation may be produced in this way by cosmic-ray-induced nuclear reactions.

Hot-atom reactions are probably responsible for the luminescence that occurs when meteors enter the earth's atmosphere (Bobrovnikoff, 1942). In Fig. 1-13*b* the lowest electronic curve (hypothetical), corresponding to the ground states of both atoms, is drawn. If the unexcited atoms are accelerated to very high approach velocities, the configuration point will rise on the lowest curve to the crossing points. Depending on conditions at these points, excited thallium and excited mercury atoms can be produced at relative rates that bear no relation to the thermal distributions of populations found at thermal equilibrium. Normal collision activation would form more excited thallium than mercury atoms, but conditions at crossing points could, under nonequilibrium conditions, reverse the distribution, thus favoring greater luminescence from mercury radiation than from that of thallium. The vaporized atoms from meteor surfaces are hot atoms with respect to the constituents of the atmosphere and probably produce the well-known radiation of meteors passing through the upper air. As in other such luminescence processes, the relative intensity of lines will, in part, be determined by conditions at crossing points.

We have mentioned that the theories of Kallmann and London and others for electronic-energy exchange under conditions of very close resonance predict large distances of particle separation over which transfer may take place. Indeed there is a variety of experiments indicating the validity of these theories. Förster (1949) found that irradiation of tryptophan in methanol solution sensitized the fluorescence of rhodamine B molecules. When the molecules were, on the average, 70 Å apart, 50 per cent of the energy was lost in this way. There is no evidence that the process is viscosity-dependent; however, as we have seen, lack of such a dependence does not establish the fact that the participants in quench-

ing are at the average distances calculated from concentrations or predicted by the very large cross sections for effective collision which would result from the application of simple collision theory. It must be suggested that the two types of molecules may form a weak complex preceding excitation.

Watson and Livingston (1950) found that quanta absorbed by chlorophylls a and b could exchange electronic excitation energy over large distances. Other experiments on such processes as the depolarization of the fluorescence of dye molecules also point to electronic-energy migrations over very large distances (Lewschin, 1924, 1935a, b; Pringsheim and Wavilov, 1926). These phenomena are not to be confused with reemission and reabsorption of light (inner filter effect), which can, for geometrical reasons, become important only at high dye concentrations. The theories that have been applied to these and other observations attribute the energy migration to the long-range coupling of dipole and higher-moment electric and magnetic fields, thus producing the necessary interaction energy for crossing of potential surfaces at large intermolecular distances. The phenomena may resemble the internal-conversion process of nuclear physics in which coupling between the nuclear fields and the field of external electrons allows excitation of the electron system during nuclear decay. Arnold and Oppenheimer (1950) have employed the comparison to calculate maximum distances for such coupling among the photosynthetic pigments of *Chroococcus*. They find a distance between 7 and 35 Å. Other theories indicate larger distances. The classical theory was given by J. Perrin (1924, 1927), and its quantum analogue by F. Perrin (1932) and by Kallmann and London (1929). Stueckelberg (1932) developed a general theory that includes the migration of electronic energy (see also Mott and Massey, 1949; Vavilov *et al.*, 1949). The most complete treatment is that of Förster (1948). All authors give primary consideration to dipole-dipole interaction, since this is the most important type. Transfers dependent on this kind of coupling correspond to excitation by electromagnetic fields and hence must satisfy the same selection rules. Only those systems in which the fluorescence band of the primarily excited molecule overlaps the absorption band of the quencher experience efficient transfer. For example, the potential-energy surface for chlorophyll a excited, chlorophyll b unexcited must overlap that for the reverse distribution of excitation energy. The further condition is that there must exist sufficient interaction through field coupling to produce the actual conditions for crossing. The interactions that are postulated are large at small intermolecular distances, analogous to van der Waals' forces that depend on the same field components. However, they fall off rapidly with distance, and it is surprising that even the relatively long-range dipole fields can interact with any strength at distances greater than a few kinetic-theory diameters. Nevertheless

theory and experiment are in agreement that energy transfer does take place in this way over very long distances. Possibly further investigation of the cases cited will demonstrate that these phenomena are artifacts, perhaps due to molecular association. There seems no reason to doubt that the coupling of electric fields is an adequate vehicle for energy migration over small distances, as found by Arnold and Oppenheimer (see Sect. 6).

Coulson and Davies (1952) have calculated, using rather extreme approximations, that the dispersion forces between conjugated molecules may fall off as slowly as  $r^{-3.5}$  at small distances of approach. Even at separations greater than a few molecular diameters, the force law is proportional to  $r^{-5}$  rather than to  $r^{-6}$ , as calculated for small or nonconjugated molecules. Molecules observed to be efficient in resonance transfer processes are of the conjugated type, and the new force law makes their high efficiency at short distances more understandable. Unfortunately the calculation sheds no light on the processes at large separation distances.

Franck and Livingston (1949) and Förster (1948) have discussed the application of this type of transfer to a variety of phenomena. For instance, the former authors attribute the quenching of anthracene fluorescence in the crystalline state by naphthacene (Winterstein and Schön, 1934; Bowen, 1938, 1944, 1945; Bowen and Mikiewicz, 1947; and others listed in Franck and Livingston, 1949) to field coupling.

The theory of another type of direct transfer of electronic energy was developed by Frenkel (1931a,b, 1936) and Peierls (1932) (see also Franck and Teller, 1938). In many crystals, and especially in polar crystals, the crystal elements are so closely spaced that the Heisenberg uncertainty principle becomes an important consideration and the crystal as a whole takes on some of the electronic properties of a single molecule. The potential-energy structure of a single element is repeated for each element of the same type but with slight perturbations that lead to the familiar band structure of allowed electronic states. So close are the energy levels in these bands that excitation energy can readily pass to distant elements. Because little atomic displacement accompanies the wandering of excitation, energy may remain for only a very short time, perhaps  $10^{-15}$  sec, at any one element. Longer periods are possible when weaker coupling exists. Heller and Marcus (1951) suggest that the weak interactions of induced dipoles in the elements can also support "exciton migration," as the process is called. Mott and Gurney (1948), who employ these exciton movements in their theory of the photographic process, liken the phenomenon to the simultaneous movement of an electron and a positive hole through the crystal lattice. Exciton movements are usually associated with the presence of new spectral bands, and this fact has led to the identification of excitons in the polymolecular associ-

ation complexes that some pseudoisocyanine dyes form in solution (Jelley, 1936, 1937; Scheibe, 1937; Scheibe *et al.*, 1939; Sheppard, 1942; Mattoon, 1944). West and Carroll (1947, 1951) have attributed the sensitization action of dyes in photographic emulsions to exciton movements through regular piles of these molecules on silver halide crystals. To be useful, the exciton must be trapped in the halide. Apparently, nonplanar dye impurities, which probably possess conditions favorable for internal conversion, become supersensitizers when they trap excitons at the halide surface. They act as antisensitizers when trapping takes place at a position in the dye pile other than at the crystal surface.

A third possible method for direct transfer of energy between electronic degrees of freedom depends on charge transfer. The phenomenon is represented on potential-energy surfaces in exactly the same way as any process in which electronic quantum numbers change. During a crossing process an electron moves from one chemical entity to another, and if these levels are separated in energy, the difference is transported. Theoretical treatments are similar to those for electronic-quanta transfer, though the interaction functions used to calculate the resonance energy are different. Application of Stueckelberg's theory (1932), which predicts long-range quanta transfer via field interaction, predicts considerably shorter distances for maximum efficient electron migration. Electron exchange between atoms and ions moving at high relative velocities has been well studied (Keene, 1949; Mott and Massey, 1949). At lower velocities, in addition to Stueckelberg's treatment, that of Kallmann and Rosen (1930) is appropriate, though both are unsatisfactory for polyatomic molecules (see also Mott and Gurney, 1948).

Charge transfer unquestionably plays an important part in the mechanism of many reactions. We do not propose to review the many publications dealing with such mechanism in nonphotochemical reactions. For photoreactions Weiss (1935, 1939a,b, 1942, 1946) (see also Franck and Levi, 1935) has proposed electron transfer as the basic mechanism in photoreductions and has extended the theory to photooxidation reactions and to quenching without net charge transfer. In photoreductions, according to the theory, the excited molecule loses an electron to the quencher. Consider, for instance, the self-quenching of anthracene, which may proceed in the following way on the potential surfaces of Fig. 1-15: One molecule of anthracene is excited to an upper state, perhaps ultimately a triplet state, from which it readily loses an electron to a second anthracene molecule. The two ions are attracted to each other and form the metastable dimer, which is returned to the ground state of two monomers via the left-hand crossing point with activation energy  $E_0$  (Bowen and Norton, 1939; Byk, 1908a,b; Weigert, 1908; Lauer and Oda, 1936; Kautsky *et al.*, 1933). Dimer or polymer formation is a common result of quenching processes, but in numerous instances these

products are due to subsequent reactions of atoms or radicals produced in the primary process (Lind and Livingston, 1930, 1933; Norrish and Griffiths, 1928; Thompson, 1939; Pringsheim, 1939).

Quenching of fluorescence of polycyclic hydrocarbons, chlorophyll, and similar molecules by molecular oxygen or nitric oxide (Bowen and Williams, 1939; Weil-Malherbe and Weiss, 1942, 1943, 1944; West and Miller, 1940) is an example of reactions probably explained by Weiss's theory. Excitation of conjugated molecules in the visible can reduce the

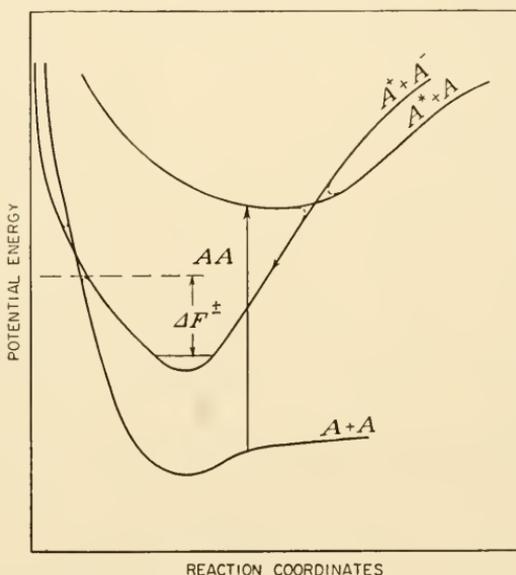


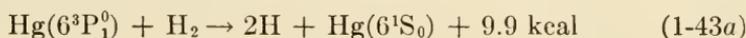
FIG. 1-15. Possible potential surfaces, based on Weiss's theory, for the mechanism of self-quenching of anthracene.

energy required to remove an electron from an average ionization potential in the ground state of 9 to 6 eV (Sugden *et al.*, 1941), so that these molecules on excitation can become very strong reducing agents, thus suggesting the validity of Weiss's ideas in these cases. Similarly the partially vacant ground state of the excited molecule can make it a strong oxidizing agent for photooxidations. Quenching without net charge loss to either participant can result from double electron migration, which restores electrical neutrality but transfers electronic excitation energy from one molecule to another. Evidence pertinent to the substantiation of the theory is considerable but not yet completely confirmatory. One striking observation bearing on the problem is that electrical conductivity increases during illumination of many quenching substances (Weiss, 1946). Rowell and LaMer (1951) have provided correlations between the relative oxidizability of excited and quenching molecules and the efficiency of quenching reactions.

## 4.4. QUENCHING WITH VIBRATIONAL-ENERGY TRANSFER

So long as electric quantum numbers alone change on collision, the transfer of energy between excited and unexcited molecules is analogous to the similar processes that have been discussed for atoms. The last part of the discussion in Sect. 4-3 dealt briefly with three rather special transfer mechanisms, all of which are supposed to depend very strongly on the extent to which the resonance requirement is satisfied. Consequently conversion of electronic energy into vibrational or external energy is not favored. Most collisions of molecules are more complicated in that many quantum numbers change during energy transfer. The simplest such collision is between an atom and a diatomic molecule, and its treatment will be adequate for collisions of larger molecules as long as we concentrate on the reactive degrees of freedom and neglect consideration of more than two vibrational quantum numbers.

Cario and Franck (1922) observed that the quenching of mercury resonance radiation by hydrogen resulted in the dissociation of the latter. The two primary steps that have received most support,



and



are reviewed by Noyes and Leighton (1941, p. 327). Proponents of the first reaction have suggested that the marked effectiveness of mercury as a photocatalyst for hydrogen dissociation is in part due to the approximate satisfaction of the resonance requirement for collisions. However, the second reaction is undoubtedly of major significance, and an energy discrepancy of 18 kcal/mole is hardly indicative of close matching of mercury excitation energy and the energy requirement of the reaction. Mercury hydride is metastable and never found in high concentration in these reactions, but that it is involved as an intermediate in the total photoreaction points up the importance of chemical affinity in quenching reactions, a fact discussed by numerous authors (Eucken and Becker, 1934; Franck and Herzfeld, 1937; Eyring, 1935). Simply on the basis of the free energy of activation, Eq. (1-43b) would be expected to proceed more efficiently. Similarly, numerous reactions in which mercury fluorescence radiation is quenched depend on the formation of metastable intermediate compounds containing mercury (HgA, HgK: Oldenberg, 1928; HgCH<sub>4</sub>: Glockler and Martin, 1934). Similar examples involving larger molecules have been illustrated by the quenching of anthracene fluorescence (Sect. 4-3).

A number of small molecules are effective in deactivating mercury atoms from the  $6^3\text{P}_1^0$  level to the metastable level  $6^3\text{P}_0^0$  by causing the redistribution of 5.01 kcal/mole in nonelectronic degrees of freedom. A

typical set of data due to Zemansky (1930) indicates quite clearly a resonance dependence of the quenching efficiency (Fig. 1-16). Other observations on similar processes verify the requirement (Mitchell and Zemansky, 1934), though Laidler (1942b) has proposed that the relation is artifactual. One is faced with the problem of reconciling the distinct chemical nature of quenching reactions with the existence of a resonance requirement. Consideration of the forms of potential surfaces involved

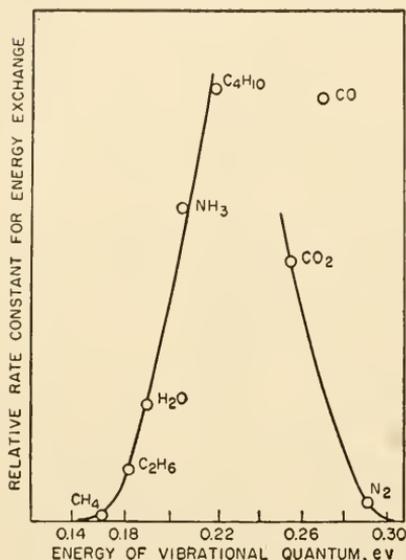
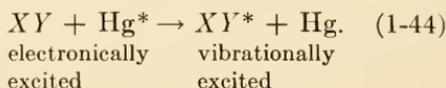


FIG. 1-16. Variation in efficiency of conversion of electronic energy into vibrational energy as a function of the size of vibrational quanta. The abscissa values are the energies of the vibrational quantum of each molecule which lies closest to the energy available in electronically excited mercury atoms. (Zemansky, 1930.)

Line III is the line of points common to both surfaces and hence includes all the collision gateways that will allow energy transfer in crossing to the lower surface. A possible projection of this line on a vertical plane such that the abscissa is the length along III is shown in Fig. 1-17d. However, each special case must be examined to determine if line III lies as shown or at a different inclination to the abscissa, perhaps positioned very near the origin, as shown in line IV of Fig. 1-17a. In any case, the line probably has one minimum of energy more or less centrally located, so that the most efficient transfer processes find their gateways toward the center of the region of maximum distortion of equipotential lines. For the present example the best gateway from the point of view of collision

in quenching reactions suggests that the data may be explained in the following manner: Figure 1-17a is a possible contour map in configuration space for the energy transfer



The equipotential lines shown in this figure apply to a single electronic potential surface for the system. Beneath it one must imagine a very similar surface for the system  $XY$  plus  $\text{Hg}$  (unexcited). At large  $\text{Hg}-XY$  distances the surfaces will be identical but separated by the excitation energy of  $\text{Hg}$ . Figure 1-17b, which is a vertical cross section through I, demonstrates the latter fact. A cross section at II (Fig. 1-17c) shows that the surfaces begin to take on different shapes as the distance of separation  $\text{Hg}-XY$  becomes smaller. There may even be a crossing point where the line II intersects the loci of crossing points III.

energy will lie on plane V of Fig. 1-17a, shown in the vertical cross section, Fig. 1-17e. The entire collision path can be projected on a vertical plane parallel to the abscissa of Fig. 1-17a, as shown in Fig. 1-17f. The latter figure is similar to the collision trajectories previously employed for quenching by atoms, and, like that case, the rate constant may be expressed by absolute theory thus:

$$k = \frac{kT}{h} t \frac{\left(\frac{2\pi m^{\ddagger} kT}{h^2}\right)^{3/2} \frac{8\pi^2 I^{\ddagger} kT}{h^2} \left(\prod_{i=1}^3 \frac{1}{1 - e^{-h\nu_i^{\ddagger}/kT}}\right) e^{-E_0/RT}}{\left(\frac{2\pi m_A kT}{h^2}\right)^{3/2} \left(\frac{2\pi m_{XY} kT}{h^2}\right)^{3/2} \frac{8\pi^2 I_{XY} kT}{h^2} \cdot \frac{1}{1 - e^{-h\nu_{XY}/kT}}}. \quad (1-45)$$

The term  $t$  contains, in addition to the crossing probability  $2p(1-p)$ , a factor to exclude those points of III at which crossing will not satisfy the quantum restrictions for the particular vibrational state on the lower slope. Only those collisional configurations on line III which have just the relative momenta to produce allowed components of vibratory motion in the HgX-Y direction will be successful in quantum transfers. Crossing with energy just  $E'_0$ , the minimum value, as shown in Fig. 1-17e, would be successful if there existed a vibrational energy level at 1 on the lower surface. If, instead, the level lies at 2, for which the original electronic energy is excessive by the amount  $E_t$ , the collision path would have to be angled toward line II in such a way that the energy discrepancy  $E_t$  is diverted into translation. Similarly, if the vibrational level in the lower state lies at 3 in Fig. 1-17e, so that the electronic energy is inadequate, the extra energy  $E'_t$  must be obtained from an external degree of freedom in order to satisfy the requirements of the lower state. The extra energy must come from increased violence of collision and so will appear as an additional increment in the collision energy  $E_0$ . Thus, if there are no other complications, the probability of excitation of vibration would be expected to fall off roughly as  $e^{-E'_t/RT}$ .

On the other hand, in the case where the quanta of the quenching oscillator fall more and more in magnitude below the electronic quanta that are to be quenched, the successful trajectories must cross line III nearer the axis along the right-to-left valley bottom (see Fig. 1-17a). This reduces the resultant vibrational motion across the valley. Such a crossing will involve an increase in the collision energy roughly proportional to the energy excess  $E_t$ , since the potential energy of line III will tend to rise as the valley axis is approached (Fig. 1-17d). Again, failure to match energies because of vibrational quanta being too small should cause an exponential drop in rate constant with energy discrepancy. Thus, all other things being equal, the rate constant for energy transfer when plotted against vibrational frequency should produce a sharply

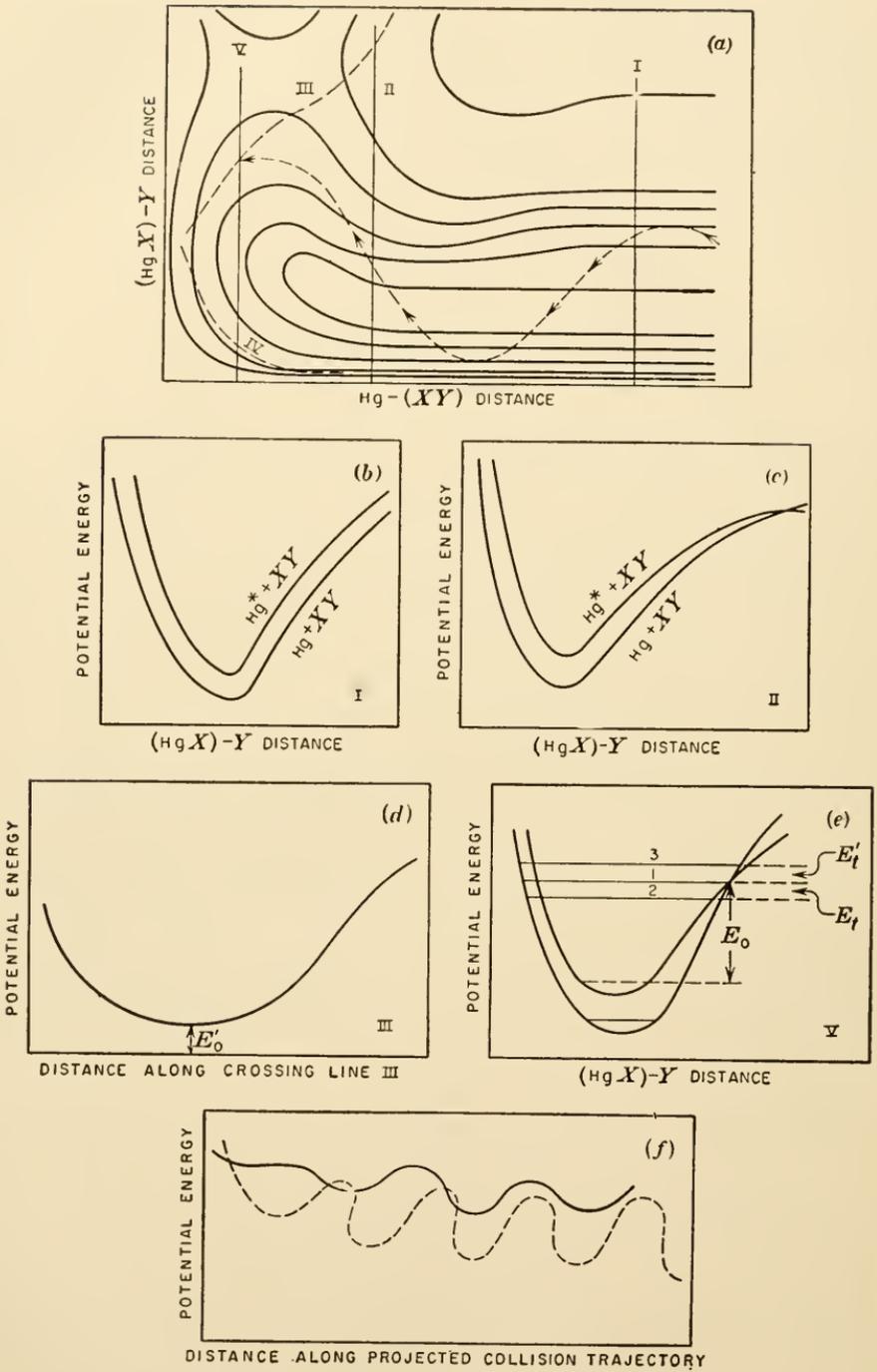


FIG. 1-17. For descriptive legend see opposite page.

peaked curve simulating a resonance curve. Compare for instance Fig. 1-16, the data of Zemansky plotted in this fashion, with Fig. 1-14.

From the model and the expression for the rate constant, one readily sees that the relation can become complicated when the different vibrational frequencies lie in different molecules. For example, the transmission coefficient or the potential-energy curve associated with section III may change markedly as the quenching oscillator is changed. The left side of Fig. 1-16 is characterized by primary collisions between mercury and hydrogen atoms of the quenching molecules, so that the various potential-energy diagrams for the different substances may be expected to be quite similar, as the data indicate. It is not probable that the plot of vibrational frequency against quenching efficiency will show the highest efficiency at the vibrational frequency that just matches in energy the electronic excitation energy. Indeed, in view of the numerous factors that establish the collision requirements, it would be surprising to find as good agreement as was found by Zemansky.

Pressure broadening (Lorentz and Holtzmark broadening) of absorption spectra is similar to the quenching of fluorescence in many ways (see, for instance, the discussion in Mitchell and Zemansky, 1934), though the effectiveness of various substances in the two processes is not parallel. The two phenomena, as well as a variety of luminescent processes, provide data for the study of diabatic collisions and are included in the domain of the present theory.

The "cracking" of highly excited molecules which occurs under electron bombardment in mass spectrometers provides interesting examples of intramolecular diabatic processes. Hydrocarbons excited to high energy states during ionization pass through many crossing points in a single oscillation of the atoms. We have previously seen that the density of crossing points will be very high. It appears probable that the ion remains in any single state such a short time that the vibrational degrees of freedom for that state never become fully developed. Consequently the ion, moving as an isolated entity through the spectrometer, will dissociate only when it finds itself simultaneously in a state in which dissociation would normally occur and with the atoms of the dissociating

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FIG. 1-17. Potential-energy diagrams illustrating a mechanism by which a resonance relation may be preserved for electronic-vibrational transfers of energy. (a) Contour map of the upper electronic surface. (b) Vertical cross section through I illustrating nearly identical noncrossing Franck curves for upper and lower electronic surfaces. (c) Vertical cross section through II illustrating difference in form of upper and lower Franck curves at smaller Hg-(XY) distances. (d) Variation in collision energy  $E_0$ , with distances along the line of crossing points, line III.  $E'_0$  is the minimum collision energy along this line. (e) Vertical section through line V showing crossing of Franck curves for upper and lower surfaces at the best gateway. (f) Collision trajectory projected on a vertical plane. The dotted line is that part of the trajectory lying on the lower surface.

bond well separated to approximately the distance required in the activated complex for this particular dissociation reaction. Under these circumstances the lifetime in that state may be great enough to permit passage over the reaction barrier to dissociated products. It is observed that the ion usually dissociates at that bond which is weakest in the ground state. Absolute-rate theory has been applied to this complicated process on the well-founded assumption that the migration of the ion among electronic-vibrational states is sufficiently rapid to produce the state of the activated complexes with a probability equal to the statistical-mechanical probability of occurrence of activated complexes in an equilibrium situation (Wallenstein *et al.*, 1951; Rosenstock *et al.*, 1952). The processes have several novel features from a rate-theory point of view. Single ions with fixed energy form the reactants, thus necessitating the use of a microcanonical ensemble in calculating probabilities for various states. For any given excited ion there exists a variety of processes with very nearly the same energy requirement relative to the total excitation energy. All the processes must be included at each step, and all steps of the cascade of decompositions, as the parent ion becomes successively degraded, must be considered.

Quantitative application of the absolute-rate theory using potential-energy surfaces in configuration space is complete if the transmission coefficient is calculated quantum-mechanically. Even without such refinements it is as complete as collision theory. It would appear profitable to apply the theory to a wide variety of collisional processes in the same way that absolute-rate theory has been applied to chemical reactions. Speaking conservatively, absolute quantitative application would be an ambitious undertaking for the simple collisions here discussed. Detailed numerical investigations of the theory for collisions involving polyatomic molecules are out of the question. Nevertheless even the more complicated energy-transfer processes of these molecules are described in a useful qualitative manner if attention is fixed on the two degrees of vibrational freedom exchanging energy. The presence of other vibrational modes will generally increase the restrictions on successful collisions for diabatic as well as adiabatic energy-transfer processes.

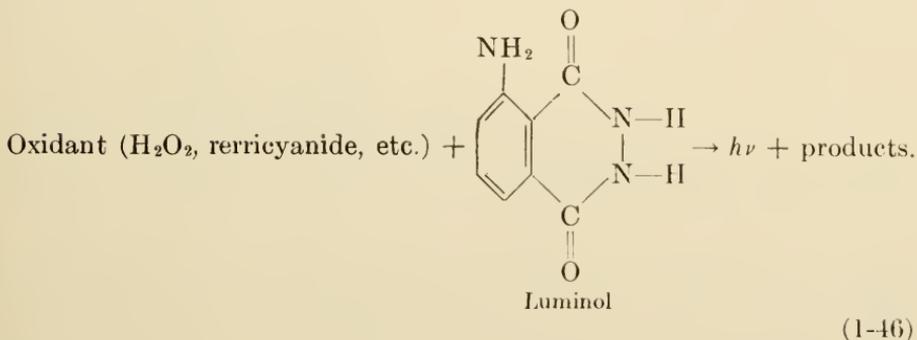
## 5. CHEMILUMINESCENCE

The principle of microscopic reversibility which applies at thermal and radiation equilibrium requires that the processes of energy transfer discussed in preceding sections be reversible, thus producing radiation from chemical, electrical, mechanical, or thermal energy. Under the non-equilibrium conditions of rate processes, probability considerations are generally unfavorable to high yields of luminescence. We have already briefly discussed thermally induced luminescence in Sect. 1-3 on Black-

body Radiation. Black-body radiation probably plays no significant role in biological processes except in the very general sense in which it controls the temperature of the earth's surface. It has been suggested that olfaction is the result of absorption by smelled substances of the black-body radiation from nerve endings (Beck and Miles, 1947). Mechanically produced luminescence has been noted, in passing, in the discussion of meteor light (Sect. 4-3). In this section, chemiluminescence need be given but summary treatment in view of the exposition of the preceding sections.

Chemiluminescence has figured in biological research primarily in the investigations of fireflies and certain luminescent algae and bacteria. It plays a considerable role in the survival ability of these organisms, and much has been learned about the behavior of proteins by its study. Harvey (1940), Drew (1939), Johnson (1947), and others have put the chemiluminescence of various organisms to good use. The luminescence intensity gives a direct measure of the velocity of certain enzyme-catalyzed reactions in the intact organism, permitting the *in vivo* study of the effect of a variety of substances and physical conditions such as pressure and temperature on these processes. The reader is referred to the more complete discussion given in a book by Johnson *et al.* (1954).

Numerous organic molecules react with oxidizing agents to produce intense chemiluminescence:



Indeed, Audubert (1936), using very sensitive light-detection equipment, observed that light is produced during the course of a number of chemical reactions not usually considered to be luminescent. Some examples are neutralization of strong acids by strong bases, oxidation of glucose by permanganate, potassium sulfate reaction with oxygen, sodium amalgam reaction with water, and the oxidation of ethanol by chromic acid. The question of the existence of natural rays (mitogenetic radiation) excited fierce controversy for a number of years. It is probable that at least some of the positive results reported were due to the type of reactions studied by Audubert and hence to normal chemiluminescence.

According to Evans *et al.* (1938), two general types of potential-energy diagrams are responsible for chemiluminescence. They proposed a diagram of the type of Fig. 1-18 for the chemiluminescent decomposition of sodium azide (Audubert, 1937). Luminescence occurs in transition 2. Reaction on the upper curve will be important only if the energy required for excitation to that surface  $E_1$  is small and the activation energy for the process on the upper surface is small with respect to that on the lower surface, i.e.,  $E_{0,e} > E_{0,\mu}$ . Though reaction via the upper surface may be a relatively poor process, a considerable intensity of luminescence may be caused by the small amount of reaction which does take place on that

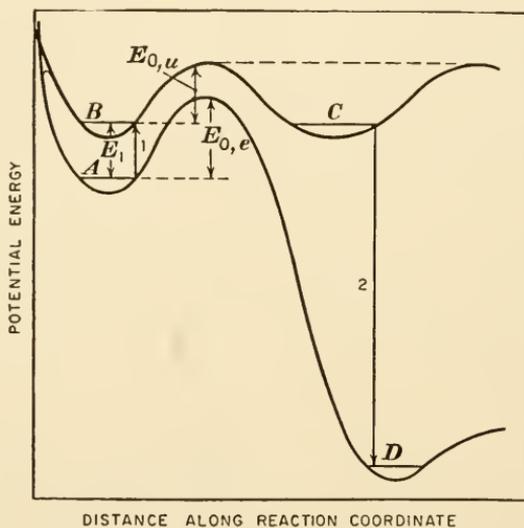


FIG. 1-18. Typical potential-energy curves for a chemiluminescent reaction occurring via black-body or collision excitation.

surface. Excitation to the upper surface can occur through absorption of black-body radiation or through collisions, but we have seen that the latter processes will be more efficient by a factor of about  $10^5$  (Sect. 1-3).

A more probable mechanism whereby chemical energy is converted into light can occur in the second kind of potential-energy diagram, typified by Fig. 1-19. Activation to the upper surface via path 1 is highly improbable, but the crossing conditions at the top of the potential barrier I on the lower surface may, under a large variety of conditions, favor migration of the configuration point to the upper surface during chemical reaction (Sect. 2-1). The intensity of luminescence via path 2 will, of course, depend on the transmission coefficient for crossing at I and the height of the potential barrier II on the upper surface. Undoubtedly the majority of Audubert's observations are explained by the existence of potential-energy surfaces, such as shown in Fig. 1-19 for the reactions he studied.

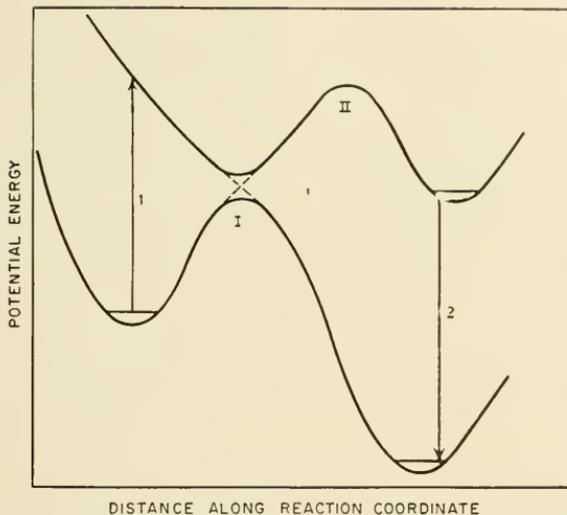


FIG. 1-19. Typical potential-energy curves for a chemiluminescent reaction occurring as the result of a diabatic process at the top of the ground-state potential barrier.

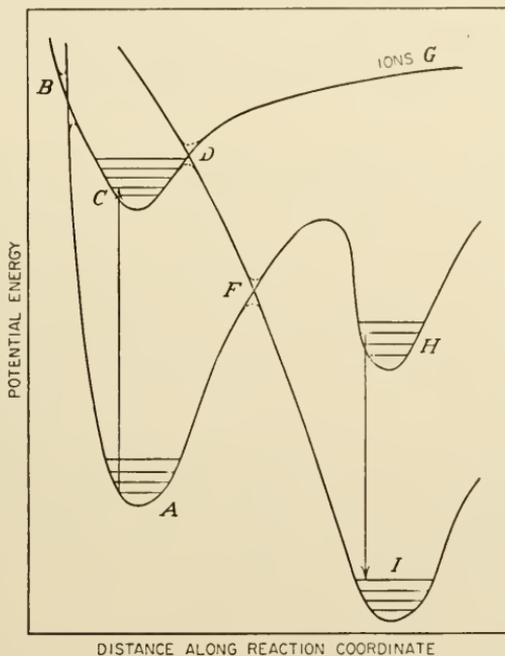


FIG. 1-20. Arrangement of potential-energy surfaces to explain anomalous radiation intensities and ion populations.

Most flames show chemiluminescence. The usual spectroscopic photograph shows very bright lines or bands superimposed on a background of black-body radiation. The bright lines prove that the corresponding high levels are much more densely populated than they would be in an equilibrium system at the same temperature. Also flames or any rapidly

reacting system show a much higher population of electrons than would correspond to black-body radiation (Thomas and Eyring, 1951). The concentration of such highly populated states depends upon the potential-energy curves for the system. Using the simplified diagram of Fig. 1-20, the anomalous populations of upper states may be explained in the following way: Most molecules pass by radiationless transitions from *A* to *I* by way of *F*, liberating energy equal to the amount by which state *I* lies below *A*. This heats the system and correspondingly promotes the black-body number of molecules in all states. However, unreacted molecules at state *A* also have the choice of passing to *H* and thus greatly overpopulating the latter state as compared with the black-body number of molecules arriving from *I*. The result is a strong chemiluminescent band due to the transitions from *H* to *I*. In the same way, molecules may pass by radiationless transition from *A* by way of *B* to the ionic state *G*, or they may pass through *C* by absorbing radiation. As a result of these and analogous processes, the ionic population will greatly exceed the number that equilibrium theory would lead one to calculate as arising from *I*. The population of the ions in the exhaust of hot flames from jet engines (Sanger *et al.*, 1949) could be readily calculated if the detailed surfaces were known. No new principles are required to calculate the statistical behavior of systems on known surfaces.

## 6. ENERGY TRANSFER IN BIOLOGICAL REACTIONS

Since molecules behave the same way in the same local environment whether within or without a living organism, we may expect to understand energy exchange in biological reactions in terms of the discussions already given. The very large molecules of biological importance—viruses, proteins, genes, and structural substances—give some possibility for anomalous behavior simply through the complexity of their size and their internal linkages. Of those mentioned, only proteins are at all well studied, and the literature of protein investigations affords only a very few clear-cut examples of surprising behavior in energy transfer. Furthermore none of these examples has been adequately investigated, and it would be out of place to discuss them in any detail. Three cases will be mentioned briefly, two of which are of special interest in this chapter because they involve photoreactions.

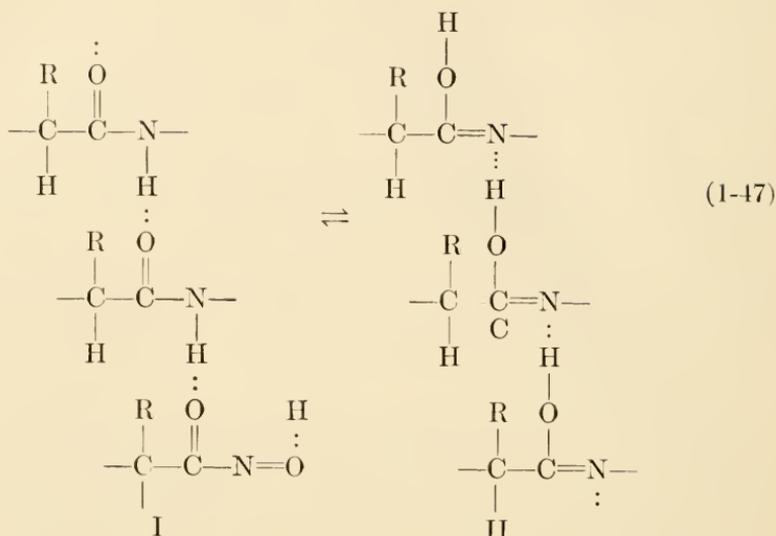
Carbon monoxide is liberated in unit quantum yield from carbonyl-myoglobin, independent of wave length in the region from 2800 to 5460 Å (Bücher and Negelein, 1942; Bücher and Kaspers, 1946). In the visible region of wave lengths the light is entirely absorbed by the prosthetic group of myoglobin, which is an iron-porphyrin, or heme. Carbon monoxide is bound at the iron atom of the heme. At 2800 Å, 50 per cent of the absorbed radiation is taken up by tyrosine and tryptophan amino

acid residues, of which there are two and four, respectively, in one molecule of myoglobin (Wyman, 1948). If these residues are randomly distributed in the protein, some mechanism must act to convey at least 15 per cent of the energy of each absorbed quantum to the heme group. Franck and Livingston (1949) have suggested that this mechanism is a direct transfer of electronic quantum through field coupling à la Förster (Sect. 4-3). On the other hand, if the absorbing residues are localized in the immediate neighborhood of the heme, energy transfer may be accomplished by the migration of vibrational quanta. Under the latter circumstances, Weiss's mechanism (Sect. 4-3) is also a possibility. Other direct processes for the transfer of electronic quanta are probably disallowed by the fact that the amino acid residues show the same spectra in and out of the protein, thus indicating the absence of strong electronic coupling among them.

Szent-Gyorgyi (1947) has discussed a series of experiments carried out by his coworkers in which dried gelatin suspensions of various dye molecules, such as eosin W and rhodamine B, manifested phosphorescence and a severalfold increase in electrical conductivity on illumination. Phosphorescence was observed when gelatin was replaced by other pure proteins. This behavior is usually associated with the existence of bands of upper, unfilled electronic states characteristic only of tightly bound crystals. Jordan (1938) and Möglich and Schön (1938) have proposed that these bands occur in single molecules of viruses, genes, and proteins. On the usual theory of photoconduction (Seitz, 1940), electronic energy would be made available at most parts of the protein by electron migration following excitation to a higher-lying band. There does not appear to be any reason to expect these bands in protein molecules, which depend for much of their configuration on weak hydrogen bonds and van der Waals' interactions. Similar considerations mediate against an explanation based on exciton migration or any other of the special processes we have discussed.

The final example of possible anomalous behavior involves electron migration and energy transfer among the various cytochromes. The action of these pigment-protein substances as intermediate catalysts and oxidizing-reducing agents in oxidative metabolism depends on their mutual linkage in a well-ordered sequence. Since the cytochromes are frequently found rigidly suspended in particles of inactive material and since the heme group of at least one member of the sequence, cytochrome C, appears to be buried in protein, as determined by its lack of chemical reactivity, the question has been raised as to the energy linkages that make functioning possible (Evans and Gergely, 1949; Geissman, 1949). If the need for a special mechanism continues to be demonstrated by further experiments, quite possibly the following explanation suggested by Geissman's work may apply: Suppose there exists a complete chain of

peptide bonds linked by hydrogen bonds as in I:

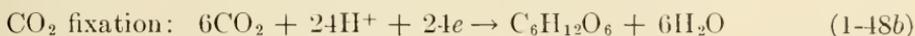
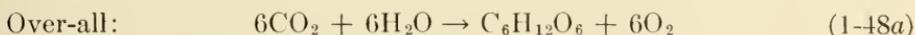


An effective electron transfer from one end of the chain to the other occurs when the tautomer II is produced by the migration of all linking protons in the chain from the equilibrium bonding position near nitrogen to that near oxygen. In this way an electron delivered at some terminal group attached to the chain could result in an electron being made available at the other end of the chain near the heme group of cytochrome C. This general idea has been indicated in the work of several authors, including Huggins (1943), Denbigh (1944), Wirtz (1947), and Schmidt (1947). These authors have, in addition, suggested the possibility that forms I and II are companion resonance structures of a conjugated system, there being a possibility in this way that an oxidized or reduced form of the protein could be produced with low activation energy. Stabilization of ionic forms of protein molecules through the existence of many resonance structures could thus perhaps explain the catalytic activities of enzymic proteins. However, Evans and Gergely (1949) showed that the overlap of electronic orbitals across hydrogen bonds is insufficient to allow resonance. Geissman has favored a resonance-like oscillation of hydrogen atoms across the interpeptide linkages, but this phenomenon would, of course, produce negligible resonance stabilization and is very unlikely to occur.

The lack of precise chemical information about proteins has led to a rash of special theories to explain the very high efficiencies of enzymes in catalysis. Among the most reasonable of these is that which may be compounded from the work of Michaelis (1946), Kalckar (1946), and LuValle and Goddard (1948). The mechanism applies to oxidative

enzymes and depends on the transient transfer of electrons to or from the protein. Only surface groups of the protein are considered to be involved in catalysis. Another interesting mechanism would depend on the folding and unfolding of the protein, during which the substrate molecule is literally torn apart (Lumry and Eyring, 1953). Although this idea stems in various forms from the early days of protein chemistry, it has recently been given quantitative support by Casey and Laidler (1950). An interesting mechanism containing both types of effects has been proposed by Smith (1949).

There is abundant evidence of unusual energy migration in photosynthesizing organisms. Modern evidence indicates that the over-all reaction of photosynthesis may be broken into two major subdivisions thus:



From the viewpoint of photochemistry the oxidation of water, Eq. (1-48c), is one of the most interesting of all photoreactions not only because of its high efficiency but also because there may be as many as three different intermediate oxygen-hydrogen compounds, all produced by the action of the same light-absorbing system. The substances that carry out this complex reaction are confined to small granular bodies whose orientation and functional structure are not understood. These bodies contain a varied array of proteins, lipoids, and pigment molecules. A number of pigment molecules have been shown to participate in photosynthesis, presumably following transfer to the chemical entity that actually combines with water (Dutton *et al.*, 1941; Emerson and Arnold, 1931-1932; Haxo and Blinks, 1950; Duysens, 1951). There must exist some means for direct transfer of electronic energy among various absorbing pigments, since neither collisions of the second kind nor reemission and reabsorption can explain the observations. This conclusion is well buttressed by the fact that the *in vivo* fluorescence of chlorophyll is sensitized in high yield by several of its companion pigments (Dutton *et al.*, 1943; Wassink and Kersten, 1946). Chemical coupling, i.e., compound formation between pigments, is not indicated. The spectral differences between *in vitro* and *in vivo* pigments are slight. Förster (1947), Arnold and Oppenheimer (1950), Duysens (1951), and others have attributed the energy transfer to the interaction of dipole fields, as proposed by Förster (Sect. 4-3) (but see Franck and Livingston, 1949). The distances over which transfer must occur do not, however, need to be especially large, since the concentrations of pigments are high. Chlorophyll, the major pigment, is present in the granules to the extent of about 0.1 *M* (Rabinowitch,

1945). *In vitro* studies of quantum migration in two-dimensional "crystals" of the chlorophylls suggest that the properties of the energy pickup system in plants are probably due to resonance migration in a specially ordered pigment lattice (Jacobs and Holt, 1952).

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## CHAPTER 2

# Electronic Structure and Excitation of Polyenes and Porphyrins

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*Polyenes: The free-electron model—Emission of light—Photoisomerism—Effect of substitution—Ring-chain systems. Porphyrins: Porphin—Structure of the visible bands—Effect of substitutions—Light emission—Electron density and excitation. References.*

This chapter might be entitled: "Why grass is green, butter yellow, and blood red." It will deal with polyenes and porphyrins, the compounds responsible for these colors. These are probably the two most important classes of biological pigments.

To understand the colors requires knowledge of the electronic structure of each compound and its connection with the absorption of light in the visible and near-ultraviolet regions of the spectrum. Three principal theoretical methods of investigation have been applied to this problem:

1. The valence bond or resonance method (Pauling, 1945; Wheland, 1944);
2. The LCAO ("Linear Combination of Atomic Orbitals") molecular-orbital method (Mulliken, 1939; Roothaan, 1951); and
3. The free-electron molecular-orbital method (Bayliss, 1948, 1949a,b, 1950, 1952; Kuhn, 1948a,b, 1949a,b, 1950; Simpson, 1948, 1949; Platt, 1949, 1953b; Ruedenberg and Scherr, 1953; Scherr, 1953).

The last of these methods is the most recent, the least known, and in many respects the simplest of the three. It will be the basis of the theoretical treatment in the present discussion. A review of this method is given by Bayliss (1952), and a later bibliography is given by Ruedenberg and Scherr (1953).

The exact general correspondence between the first two methods was recently shown by Longuet-Higgins (1950a) and Dewar and Longuet-Higgins (1952). Ruedenberg and Scherr (1953) have shown an even more detailed correspondence between the last two methods. Consequently results obtained by one method are expected to be valid for all, except for numerical details.

An exhaustive bibliography of the free-electron method would run to

dozens of papers; of the other two methods, to hundreds; and of experimental measurements, to hundreds more; therefore only key references will be cited. For brevity, also, the basic principles of interpretation will be given somewhat ex cathedra, without elaborate empirical or theoretical justification. They have been discussed by Platt (1953a).

*Electronic Origin of Color.* The only organic compounds that absorb light strongly between 2000 and 10,000 Å are unsaturated. The strongest absorbers contain long conjugated chains, ring-chain systems, or ring

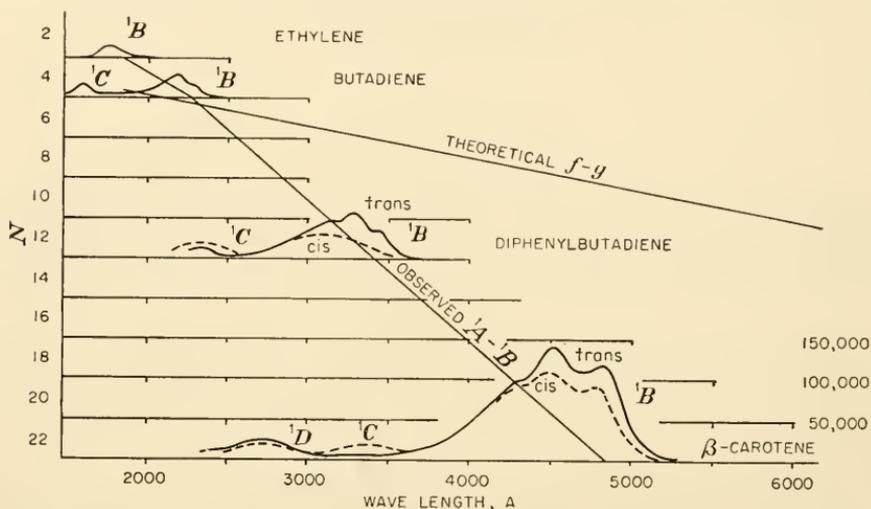


FIG. 2-1. Spectra of polyenes: ethylene (Platt *et al.*, 1949), butadiene (Jacobs and Platt, 1948), diphenyl butadiene (Pinckard *et al.*, 1948),  $\beta$ -carotene (Zechmeister and Polgár, 1943).

systems of alternating single and double bonds. The spectra are due principally to excitation of the loosely bound “unsaturation electrons” or “ $\pi$ -electrons” of such systems. In a conjugated pure hydrocarbon, each carbon atom brings one such electron to the system. These electrons are largely responsible for the chemical reactivity as well as for the light absorption of such systems, since they may be excited or removed from the molecule comparatively easily. The conjugated system is held planar by the  $\pi$ -electrons; their wave functions have a node in the plane. If it is twisted out of the plane, as by steric hindrance, the conjugation is broken, and the spectra and chemical properties are greatly altered.

We shall see later how another class of loosely bound electrons—the nonbonding electrons of conjugated hetero atoms—also contributes to the spectra, just as it does to the chemical reactivity.

### POLYENES

We begin with conjugated chains. The spectra of such systems as those shown in Figs. 2-1 and 2 have at the longest wave lengths a strong first transition whose molar extinction coefficient  $\epsilon$  is greater than 15,000.

This is followed by two or three distinct transitions of various intensities at shorter wave lengths. Empirically and theoretically, the first few strong transitions in such a spectrum have the same general properties with respect to their electron distributions, polarizations, intensities, change of intensities with cis-trans isomerism, and so on, regardless of

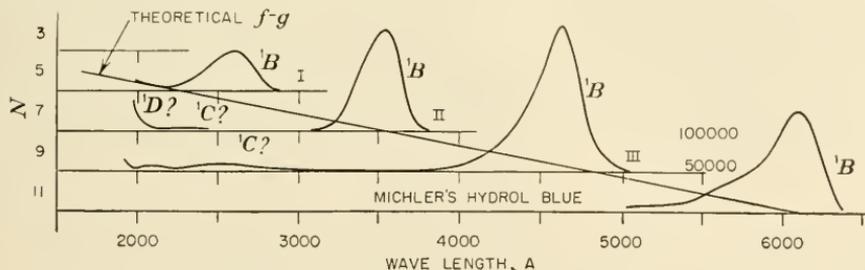


Fig. 2-2. Spectra of symmetrical amidinium ions I, II, III:



(from Simpson, 1948, and König and Regner, 1930); corrected 500 Å to violet to allow for tetramethyl substitution. Michler's Hydrol Blue. (Brooker and Sprague, 1941.)

whether the conjugated chain is a polyene, a phenyl or diphenyl polyene (or naphthyl polyene if the chain is long enough), or a cyanine dye. We may therefore use the same general theory to account for the long-wave-length spectra of all these molecular types.

### THE FREE-ELECTRON MODEL

The main features of the spectra are accounted for if we assume that the  $\pi$ -electrons are simply confined to a line running the length of the conjugated system—whether straight or zigzag does not much matter. If the atomic nuclei are equally spaced along this line, the potential field in which the electrons move will look something like the lower line in Fig. 2-3, with deep holes near every nucleus. For simplicity, we may approximate this potential by the one-dimensional "square well" shown by the straight line, with the potential constant and equal to zero along the line and rising to infinity at the ends.

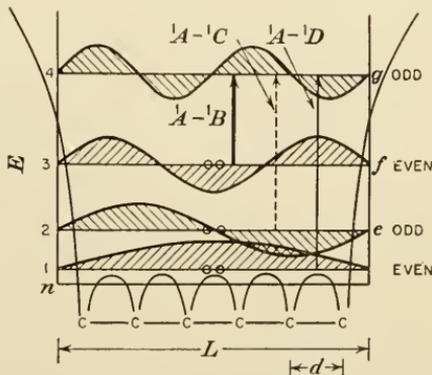


Fig. 2-3. Free-electron orbitals, energies, and transitions for hexatriene.

In such a potential well the permissible wave functions  $\psi$  are sine functions, vanishing at the ends of the trough:

$$\psi_n = \sqrt{\frac{2}{L}} \sin \frac{n\pi x}{L}, \quad n = 1, 2, 3, \dots, \quad (2-1)$$

where  $L$  is the length of the trough and  $x$  is the distance measured from one end. The normalization factor that multiplies the sine function is chosen so that the integral of the electron density (the square of the wave function) over the length of the trough,  $\int_0^L \psi^2 dx$ , will be unity.

The lowest wave function has a wave length  $\lambda = 2L$ , with no nodes in the trough; the next has  $\lambda = 2L/2$ , with one node; the  $n$ th has  $\lambda = 2L/n$ , with  $n - 1$  nodes; and so on, like the oscillations of a vibrating string.

*Orbital Energies.* The energies of these orbitals are determined by the de Broglie relation between electron momentum  $mv_n$  and wave length,

$$mv_n = h/\lambda_n, \quad (2-2)$$

where  $m$  is the electron mass and  $h$  is Planck's constant. This relation fixes the electron velocity  $v_n$ ; and the energy  $E_n$  is then

$$E_n = \frac{1}{2} mv_n^2 = \frac{1}{2m} \frac{h^2}{\lambda_n^2} = \frac{n^2 h^2}{8mL^2}. \quad (2-3)$$

The energy varies quadratically with  $n$ .

If we assume that the length  $L$  for a conjugated chain of  $N$  atoms is  $Nd$ , where  $d$  is the average interatomic distance, then

$$E_n = \frac{n^2 h^2}{8md^2 N^2} = 153,000 \frac{n^2}{N^2} \text{ cm}^{-1}. \quad (2-4)$$

The second expression gives the energy in wave numbers if  $d$  is 1.40 Å—the average C—C distance in a conjugated system.

The Pauli principle specifies that only two electrons, of opposite spin, may occupy each orbital. For a polyene where there are  $N$   $\pi$ -electrons in the lowest possible energy state, they then fill up the lowest  $N/2$  orbitals ( $N$  is even). The energy required to lift an electron from the highest filled orbital to the lowest unfilled orbital becomes

$$E_{(N/2)+1} - E_{N/2} = \frac{(N/2 + 1)^2 h^2}{8md^2 N^2} - \frac{(N/2)^2 h^2}{8md^2 N^2} \quad (2-5)$$

or

$$\Delta E = \frac{h^2}{8md^2} \frac{N + 1}{N^2} = 153,000 \frac{N + 1}{N^2} \text{ cm}^{-1}. \quad (2-6a)$$

This should represent approximately the energy of the first spectroscopic transition. For long chains it should vary as  $1/N$ . Note the absence of adjustable parameters. Values predicted by this formula are given by the slanted "theoretical" line in Fig. 2-1 for comparison with the observed first transitions (marked  $^1B$ ). The predicted wave lengths are about right for butadiene. The predictions get poorer with increasing wave length, varying about twice as fast with  $N$  as observed.

A polymethine ionic dye like those of Fig. 2-2, with  $N$  odd but with an even number of electrons, has the first  $(N + 1)/2$  orbitals filled, and

$$\Delta E = 153,000 \frac{N + 2}{N^2}. \quad (2-6b)$$

Values predicted by this formula are shown by the slanted line in Fig. 2-2 and fit the observations extremely well. [Dewar (1950) has computed the first absorption frequencies of a number of such dyes by the LCAO method. He has also predicted (1952) the polyene frequencies by an ingenious approximation based on the simple zero-energy molecular orbitals.]

The frequency  $\nu$  of the quantum-mechanical absorption is given by

$$\nu_{qu} = \Delta E/h. \quad (2-7)$$

The corresponding classical electromagnetic absorption frequency of a dipole antenna of the same length as the molecule is given by

$$\nu_{cl} = c/2L, \quad (2-8)$$

where  $c$  is the velocity of light. This frequency is lower than the frequency determined from Eqs. (2-6a) and (2-7) by a factor  $h/4mcd$ , or about  $1/230$ . This simply means that the velocities of the electrons that carry the quantum-mechanical oscillation are smaller by this factor than the electromagnetic-wave velocity, the speed of light. The wave length absorbed is therefore not  $2L$ , as it would be classically, but about

$$\lambda = 500L. \quad (2-9)$$

This linear relation between wave length and molecular length is far from exact in the polyenes, but it helps us understand the approximately constant wavelength shifts introduced by adding additional units to a conjugated chain.

The second absorption frequency of a polyene will be determined by the energy jump that is shown by a dashed line in Fig. 2-3. This corresponds to the absorption regions marked  $1C$  in Figs. 2-1 and 2.

*Allowed and Forbidden Transitions.* The lowest absorption frequency is "allowed," but the second lowest frequency is "forbidden" if the conjugated system is truly a straight line or even if it has a "center of symmetry," as it would have in a polyene in the zigzag trans, trans, . . . , trans form.

These terms have the following significance: For any molecule with a center of symmetry, there are two classes of wave functions, "even" and "odd." Even functions are given by odd values of the integer  $n$  in Eqs. (2-1) to (2-4). Such a function  $\psi$  is exactly equal to itself when reflected in the center of the molecule. The odd functions are given by even  $n$  in Eqs. (2-1) to (2-4). In them  $\psi$  is changed into its negative on reflection in the center; it therefore has a node at the center and vanishes

there. (We neglect here the additional node in the plane of the molecule common to all orbitals.)

Now, the "oscillator strength" of a transition can be found experimentally from the integrated absorption intensity,

$$f = 4.32 \times 10^{-9} \int \epsilon_\nu d\nu, \quad (2-10)$$

where  $\epsilon_\nu$  is the observed molar extinction as a function of the frequency  $\nu$  in wave numbers. This quantity is predicted theoretically by the expression

$$f = 1.085 \times 10^{-5} \nu Q^2, \quad (2-11)$$

where  $Q$  is the "transition matrix element" for one electron to jump from state  $n$  to state  $m$ :

$$Q_{x, nm} = \int \psi_n x \psi_m d\tau \quad (x\text{-component}), \quad (2-12)$$

where  $x$  is the coordinate measured from the center of the molecule in angstroms and  $d\tau$  is the volume element of integration.

The components of  $Q_{nm}$  all vanish when both  $\psi_n$  and  $\psi_m$  are even or when both are odd, and the transition is then "forbidden" (Laporte rule). Actually in a polyatomic molecule there is enough vibrational motion so that the center of symmetry is not preserved. As a result, the transitions that are believed to be of this forbidden type may still be seen, but they are only about one-fifth as strong as their "allowed" counterparts, which are transitions between even and odd orbitals.

*Intensities, Polarizations, and Molecular Configurations.* On insertion of the free-electron polyene  $\psi$ -functions into the expression for  $Q$ , it will be found that  $Q$  is largest for  $|n - m| = 1$ , i.e., for the lowest allowed transition, and that the oscillator strength falls off approximately as  $1/|n - m|$  for the higher allowed transitions.

For polyenes of different lengths,  $Q$  for the first allowed transition should be proportional to the length, and  $f$  to  $\nu Q^2$ , but since  $\nu$  varies approximately inversely with the length,  $f$  is also approximately proportional to the length. This was one of the first empirical conclusions from the comparative study of polyenes (Hausser *et al.*, 1935; Smakula, 1934). The width of the first polyene absorption region is approximately constant, and  $\epsilon_{\max}$  is therefore also approximately proportional to the length.

Intensity predictions by the LCAO method (Mulliken and Rieke, 1941) are too large, but their relative values for different molecules are accurate. Predictions using the free-electron model appear to be accurate both absolutely and relatively (Bayliss, 1948, 1952; Kuhn, 1948b; Simpson, 1948). The low allowed transitions of  $\pi$ -electrons in extended polyenes should be, and apparently are, polarized along the  $x$ -axis; i.e.,  $Q_y = Q_z = 0$ .

If the polyene has one *cis* link in the middle, so that it is doubled back on itself like a hairpin, these intensity relations are changed. The first

transition should become weaker and be polarized on a line from one tip of the molecule to the other. The second, instead of being forbidden, may become stronger than the first and will be polarized along the axis of the hairpin.

This behavior may be observed in going from the spectrum of butadiene to that of cyclohexadiene, where the conjugated system is doubled back on itself in this way (Mulliken, 1939). It was also seen in the cis-trans isomerism of longer chains by Zechmeister and Polgár (1943), Sandoval and Zechmeister (1947), and Pinckard *et al.* (1948). These authors called the transition designated as  ${}^1C$  in Figs. 2-1 and 2 the "cis band" because of its strength in this molecular configuration. The increase of intensity of a transition in the cis form is then an excellent criterion for the assignment of this transition to the forbidden class in the trans form; the loss of intensity, for its assignment as allowed. Of course, two cis bonds in a molecule, if properly placed, may restore its center of symmetry and make the  ${}^1C$  transition forbidden again, as Zechmeister found in several cases. It is illuminating to compare his fine classical interpretation of the spectra (1944) with its quantum-mechanical counterpart as given here.

*Alternation of Electron Densities.* We may use the free-electron model to determine the electron densities in a polyene. For any wave function  $\psi$ , the density is given by  $\psi^2$  if  $\psi$  is real, as it is here. In Fig. 2-4 the density is shown in the lower curves for the different  $\psi_n$ 's of hexatriene. The total  $\pi$ -electron density per unit length of the trough for  $N$  electrons in the ground state is

$$D = 2 \sum_{n=1}^{N/2} \psi_n^2 = 2 \sum_{n=1}^{N/2} \frac{2}{L} \sin^2 \frac{n\pi x}{L}. \quad (2-13)$$

The sum can be transformed into

$$D = \frac{1}{d} - \frac{2}{L} \frac{\sin \pi x/2d}{\sin \pi x/L} \cos \pi \left( \frac{x}{2d} + \frac{x}{L} \right); \quad (2-14)$$

$$= 0 \text{ at } x = 0, x = L; \quad (2-14a)$$

$$= \frac{1}{d} \text{ at } x = 2d, 4d, \dots, (N-2)d; \quad (2-14b)$$

$$= \frac{1}{d} + \frac{2}{Nd} \text{ at } x = d, 3d, 5d, \dots, (N-1)d. \quad (2-14c)$$

This curve is shown at the top of Fig. 2-4.

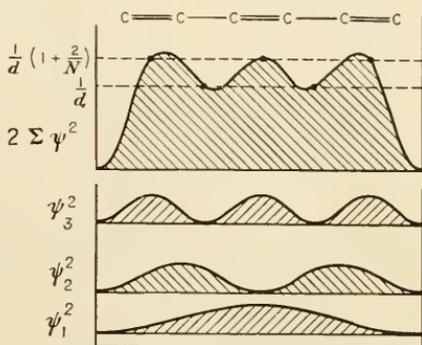


FIG. 2-4. Electron density in hexatriene on the free-electron model.

The alternating maxima and minima form a free-electron counterpart to the alternating single and double bonds in the simplest classical valence-bond diagram. Kuhn (1950) gives several examples. The average distance between maxima, or between minima, is just  $2d$ , and the maxima are located in the center of the classical double bonds. The alternation becomes less marked with increasing  $N$  and is less marked in the center of the polyene than at the ends. The maxima and minima, from the present point of view, depend on the wave character of electrons, just as do the maxima and minima of different atomic shells in the radial electron densities for an atom. The relation of these polyene density alternations to the classical bond diagrams is then something like

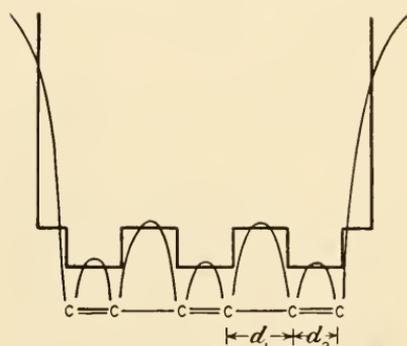


FIG. 2-5. Free-electron potential with Kuhn periodicity from alternating double and single bonds.

the relation between the positions of maximum density in atoms and the Bohr classical orbit radii.

The effect of high electron density at particular points in the polyenes is to attract the neighboring nuclei, producing a shorter bond length and higher force constant. Low density has the opposite effect, so that an alternation of electron densities implies an alternation of bond lengths and force constants. This alternation is well known from the classical valence-bond treatment, as well as

experimentally from X-ray diffraction and infrared analysis.

But from the present point of view it could be said that the alternating bond lengths give direct evidence of the wave character of electrons—almost as direct as the Davisson-Germer experiment on the diffraction of electrons from a crystal. The alternation of bond lengths shows the presence of standing waves produced by the internal interference of electron waves reflected from the ends of the molecule.

*Effect of Alternation on Transition Frequencies.* Kuhn (1950) has shown how to correct the errors of the polyene frequency predictions by introducing an alternation of period  $2d$  into the square-well potential, as shown in Fig. 2-5, using deeper minima near the classical double bonds, where the nuclei are closer together.

The effect of this periodic perturbation on free-electron and LCAO orbital energies in molecules, just as in metals (Seitz, 1940; Brillouin, 1946), is to enlarge the energy gap between the orbitals of wave lengths of more than  $4d$  and those of wave lengths of less than  $4d$ . In the language of metal theory, the periodicity introduces a Brillouin boundary between the first and second Brillouin zones. But this enlarged gap is just the energy gap corresponding to the first transition, so that this

transition can now be at higher frequencies than was predicted by the simple square-well potential, and it fits the observations better. The bottom part of Fig. 2-6 shows schematically the orbital energies of several polyenes, as observed and as calculated by the simple free-electron treat-

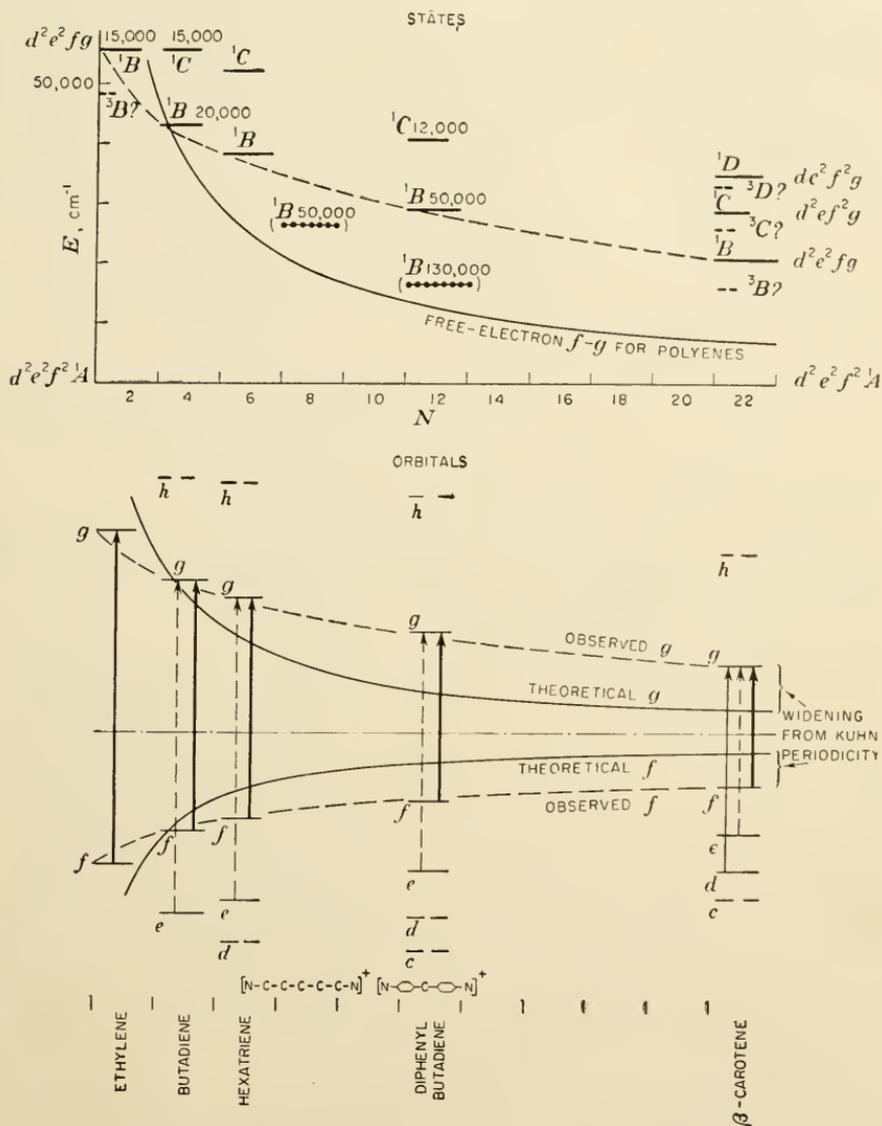


FIG. 2-6. States and orbitals of conjugated chains. The dotted energy states are for the indicated cyanine dyes. Figures over the states indicate peak extinction values.

ment, and shows how the Kuhn (1950) periodicity accounts qualitatively for the wider observed gap.

*Removal of Alternation: Cyanine Dyes.* This explanation of the surprisingly high frequencies in polyenes becomes more convincing when we

remove the alternation and see a shift to lower frequencies. Kuhn (1949b) showed how we can do this, in effect, in the symmetrical cyanine dyes, where an even number of electrons are confined to an odd-membered chain. It is then the atoms and not the bonds which are at alternate maxima and minima of electron density. The bonds are all approximately equivalent. These features have their counterparts in the classical valence structures for these molecules, where the excess charge is localized on alternate atoms and where every bond is alike, a one-and-one-half bond, single in one valence structure and double in another, equivalent structure. There is then no alternation of bond lengths. The alternation of charges on the atoms does produce a periodic potential, but a much smaller one than when adjacent atoms are drawn together. Therefore, as seen in Fig. 2-2 and in the top part of Fig. 2-6, these compounds should, and do, absorb at almost exactly the frequencies predicted by the square-well approximation.

With unsymmetrical cyanines, some alternation appears again and may increase in magnitude up to its value in the polyenes. The spectra show corresponding shifts to higher frequencies with greater asymmetry, as was shown by Brooker and Sprague (1941) and Kuhn (1949b).

Possibly the difference between the symmetrical cyanines and the polyenes will be less marked for exceedingly long chains, longer than any observed in the laboratory, because the alternation in the polyenes should eventually approach zero with increasing length.

*Nomenclature.* The molecular orbital energies shown in Fig. 2-3 and at the bottom of Fig. 2-6 were obtained by letting a single electron travel in a potential trough. Each of these orbitals or energies then represents a *shell*, in which electrons may be located, like the X-ray shells of an atom. The total energy of the molecule is approximately the sum of these one-electron energies for all the electrons. The resonance energy may be computed simply by carrying out the summation and comparing it with the energy if the molecule were separated into isolated double bonds.

The total energy of the molecule in its lowest energy state, or with various types of excitation of an electron from a filled shell to an unfilled shell, is shown in the energy-level diagram or *state* diagram at the top of Fig. 2-6. The distinction between shells and states is essential and must be remembered.

It is convenient to adopt the following simple uniform nomenclature for all conjugated systems: Let the highest filled shell be *f*, the next highest, *e*, etc.; the lowest unfilled, *g*, the next lowest, *h*, etc. The first transition will then be *f-g*, the next, *e-g*, etc.

The *configuration* of the whole molecule specifies how many electrons are in each shell. The lowest energy configuration is then . . .  $d^2e^2f^2$ ; the next lowest, . . .  $d^2e^2fg$ ; the next, . . .  $d^2ef^2g$ ; etc., where the superscripts give the number of electrons of each kind.

Each configuration gives rise to a group of states, which have slightly different energies, because of the interactions of the electrons with each other which have been neglected up to now. For polyenes, which are especially simple, each configuration has two states: a singlet in which all electron spins are paired and a triplet in which two are unpaired. An exception is the ground state and others like it in which the electrons in each shell are paired and there can be no triplet. For convenience, we may label the states *A* (ground), *B* (first excited), *C* (second excited), and so on. The configurations and states for any polyene are listed in Table 2-1. A left-hand superscript on a state shows whether it is singlet

TABLE 2-1. CONFIGURATIONS AND STATES OF POLYENES

Excitation	Configura- tion	States	Parity	Transition from ground (zigzag form)	Mulliken notation (butadiene)
.	.	.	.	.	.
<i>f-i</i>	. . . $d^2e^2fi$	$^1D_3^o$ $^3D_3^o$	Odd	Weak allowed, polarized longitudinally	Missing $V_4$
<i>e-h</i>	. . . $d^2ef^2h$	$^1D_2^o$ $^3D_2^o$			
<i>d-g</i>	. . . $de^2f^2g$	$^1D_1^o$ $^3D_1^o$	Even	Forbidden	Missing $V_3$
<i>f-h</i>	. . . $d^2ef^2h$	$^1C_2$ $^3C_2$			
<i>e-g</i>	. . . $d^2ef^2g$	$^1C$ $^3C$	Even	Forbidden	$V_2$
<i>f-g</i> (first excited)	. . . $d^2ef^2g$	$^1B^o$ $^3B^o$	Odd	Strong allowed, polarized longitudinally	$V_1$
None (ground)	. . . $d^2e^2f^2$	$^1A$	Even	.	<i>A</i>
With hetero replacements					
.	.	.	.	.	.
<i>n-g</i>	. . . $d^2e^2f^2ng$	$^1U$ $^3U$ $^1W$ $^3W$		Forbidden (very) Very weak allowed, <i>z</i> -polarized	
None	. . . $d^2e^2f^2n^2$	$^1A$			
With phenyl rings					
<i>v-w</i>	. . . $d^2ve^2f^2w$	$^1H$ $^3H$		Strong allowed, polarized longitudinally	
<i>v-g</i>	. . . $d^2ve^2f^2g$	$^1G$ $^3G$		Weak allowed, polarized transversely	
None	. . . $d^2v^2e^2f^2$	$^1A$			

or triplet. A superscript zero is placed at the right when a state is "odd," that is, when the product of orbital types, even or odd, for all electrons is odd. Only even-odd transitions are allowed. The "even" and "odd" notation is, of course, valid and useful only when a molecule has a center of symmetry. The Mulliken notation (1939; Mulliken and Rieke, 1941) for polyene states is also indicated in Table 2-1 for comparison.

Strictly, the transition frequencies predicted by the free-electron model, as just given and as shown in Figs. 2-1, 2, and 6, were computed neglecting electron interaction, and so they represent only the energy jump between shells, or the "center of gravity" of a configuration. It was therefore slightly misleading in the figures to compare the first predicted frequency with the first strong absorption frequency, which is undoubtedly  ${}^1A-{}^1B^0$ . What we should have done was to locate the other transition to the first excited configuration  ${}^1A-{}^3B^0$ , which lies at lower frequencies (by the Hund rule, which says that, in a given configuration, states of higher spin generally lie lower). The average of these two frequencies could then be compared with the predicted frequency.

However, the singlet-triplet intensity is weaker than the singlet-singlet by a factor of about  $10^5$  in hydrocarbons. For this reason and probably for other reasons discussed later under Photoisomerism, excited triplets seem never to have been found in polyenes, so that the exact location of the centers of gravity of excited configurations is not feasible. We may nevertheless use the theory for qualitative understanding or for comparing the singlet-singlet transitions of similar molecules. For these purposes our ignorance of the triplets is not so serious.

In the upper part of Fig. 2-6 the length of the horizontal lines indicates the logarithm of the molar extinction of the transition from the ground state, since this is a useful indication of the character of an excited state. For completeness the unknown triplets are indicated schematically for  $\beta$ -carotene. Such an energy-level diagram is a simple way of summing up the information on electronic excitation obtained from an absorption spectrum. It omits the vibrational structure shown by the exact contour of the spectrum, which in large molecules is not essential to an understanding of the electronic excitation.

The "free-electron" line in the top half of Fig. 2-6 shows the predicted position of the center of gravity of the first excited configuration by the free-electron model. It is seen that a symmetrical cyanine fits this curve much better than the corresponding polyene because of the alternating Kuhn potential in the latter.

#### EMISSION OF LIGHT

The emission of light by an excited molecule is a process the reverse of the absorption process. It seems to be controlled, as the absorption is not, by time-constant considerations. An energy-level diagram of a



Oscillator strengths are of more theoretical significance than extinction coefficients. They are related to the intrinsic time constants as follows:

$$f\tau = 1.5/\nu^2 \text{ for fluorescence} \\ \approx 2 \times 10^{-9} \text{ sec near } 3500 \text{ \AA};$$

and

$$f\tau = 4.5/\nu^2 \text{ for phosphorescence} \\ \approx 10^{-8} \text{ sec near } 5000 \text{ \AA}.$$

These formulas were derived from one given by Lewis and Kasha (1945) (neglecting some questionable terms due to the refractive index of the solvent) and from Eq. (2-10). A chart summarizing the known time con-

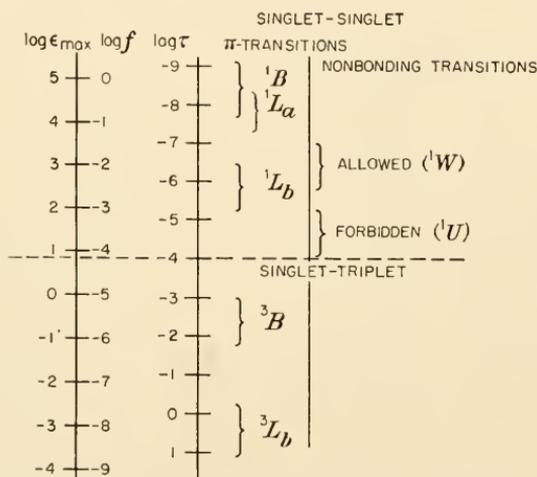


FIG. 2-8. Approximate molar extinctions, oscillator strengths for absorption from ground, and luminescence lifetimes for different types of upper states. (Compiled with the help of M. Kasha.) Nonbonding triplets are not well known and have been omitted (Reid, 1953).

stants, together with the related peak intensities and oscillator strengths for a number of types of transition in condensed-ring systems, is given in Fig. 2-8 and fits these formulas quite well. The  ${}^1B$  states of such systems are similar to those of polyenes in their properties.

But photon emission must compete with other processes. One such process is radiationless transition between excited singlets, the result of the crossing of their potential curves, which we have not discussed here (Franck and Sponer, 1948). Such transitions are indicated by dashed lines in Fig. 2-7. In these a vibrational distortion of the molecule may permit it to go from one excited state by easy stages into another in times of the order of a few vibration periods, say,  $10^{-12}$ – $10^{-13}$  sec. To go from singlet to triplet by this process is more difficult, since one of the electron spins must reverse itself at the same time. The time for this process in a hydrocarbon is more like  $10^{-7}$  sec.

The singlet-singlet radiationless process is much faster than photon emission or singlet-triplet radiationless transitions. As a result, the molecule cascades directly down from the higher excited singlets to the first one in about  $10^{-12}$  sec or less without light emission. This cascade internally quenches the fluorescence that might otherwise have been expected from these higher states.

The first excited state is sufficiently far above the ground state in most cases so that the radiationless process becomes unimportant. The molecule may then go from this state to the ground state by fluorescence ( $F$  in Fig. 2-7) in  $10^{-9}$  sec. Or it may occasionally go to the lowest triplet in  $10^{-7}$  sec and from there to the ground state by phosphorescence ( $P$ ) in  $10^{-4}$  sec (Lewis and Kasha, 1944).

*Effect of Heavy Atoms and Oxygen.* The time to go to the lowest triplet and the time to phosphoresce are materially shortened by the presence of heavy atoms such as iodine, bromine, or heavy metals in the molecule or in the surrounding medium (McClure, 1949; Kasha, 1952). This is due to the large spin-orbit coupling in heavy atoms which makes the singlet and triplet states interact more strongly; the coupling is proportional to the square of the nuclear charge. [Some complexing agents have effects like those of heavy atoms, but the explanation of this effect is not yet clear (Reid, 1952).] The result may be that the fluorescence is almost completely converted to phosphorescence, which then becomes very strong but has a short lifetime.

The presence of paramagnetic substances such as dissolved molecular oxygen or the presence of a metal atom with an odd electron, as in the copper-porphyrin complexes, may have much the same effect (Calvin and Dorough, 1948).

*Thermal Quenching.* The "intrinsic lifetimes" of long-lived luminescences can be measured accurately only at low temperatures or in solids where another competing process, "external quenching," is negligible. In solutions at room temperature the random interaction with such molecules as solvent molecules or dissolved oxygen produces a crossing of potential curves even between the first excited states and the ground state, so that the fluorescence and phosphorescence energy is dissipated into vibrations and thermal energy in times of the order of perhaps  $10^{-5}$  sec. This quenches the phosphorescence, though not the fluorescence.

*Energy Transfer.* Another type of external quenching which has received much attention lately is the radiationless transfer of energy from one excited molecule to another that has a lower-energy excited configuration (Bowen, 1938; Kallmann and Furst, 1950; Franck and Livingston, 1949; Förster, 1951; Moodie and Reid, 1952). In mixtures the light emission may come almost exclusively from the molecular species that has the lowest fluorescent or phosphorescent state, apparently even when the concentration of this species is only a fraction of a per cent. and

regardless of what other species has actually absorbed the light initially.

This "sensitized fluorescence" is a subtle source of error in luminescence measurements. Correspondingly it may be of great importance in fluorescence and quenching and in energy transfer between molecules or unconjugated parts of molecules in biological systems.

### PHOTOISOMERISM

Most excited polyene molecules therefore spend a long time, perhaps  $10^{-9}$  sec, in the first excited singlet state, and  $10^{-5}$  sec or more in the triplet state if an appreciable fraction of them get into this state. These times are long, i.e., by comparison with the time needed to execute molecular vibrations, rearrangement, or dissociation, or rotations or

Brownian motions and collisions leading to chemical reactions. The excitation energy is also ample for many chemical effects, 50 kcal/mole or more for visible absorption, and effects are easily produced for which the electron density distribution in the excited state is favorable.

An especially interesting photochemical effect is the photoisomerization of polyenes, as described in the classic studies of Zechmeister and Polgár (1943) on carotenes and in those of Zechmeister and co-workers on diphenyl butadienes (Sandoval and Zechmeister, 1947; Pinckard *et al.*, 1948).

*Ethylene.* We may begin to understand what happens by considering how the ground and excited states behave in the simplest case, ethylene, when the two ends of the

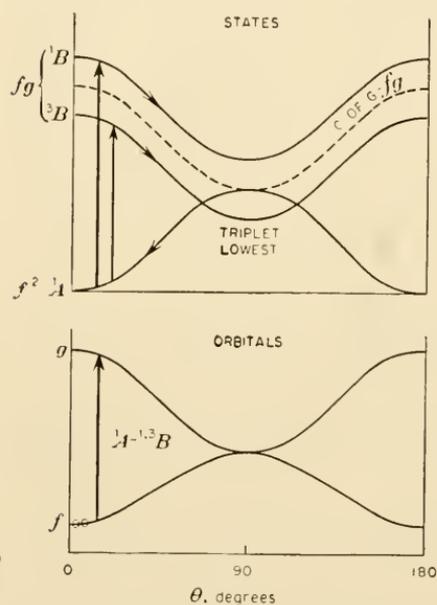


FIG. 2-9. Change of ethylene orbitals and states with angle of twist. (Adapted from Mulliken and Roothaan, 1947.)

molecule are twisted with respect to each other around the double-bond axis (Fig. 2-9).

On any orbital model whatever, a  $90^\circ$  twist in ethylene means that the orbitals we have called  $f$  and  $g$  must become degenerate (coincide). The energy of the  $f$ -orbital must have been increased, and that of the  $g$ -orbital decreased, until they are equal, as shown in Fig. 2-9. On the energy-level diagram the energy of the  $f^2$  configuration then coincides with the center of gravity of the  $fg$  configuration, and the lowest state of the

molecule is the *fg* triplet  $^3B$ . Numerical calculations by the LCAO molecular-orbital method first gave this result for ethylene (see Mulliken and Roothaan, 1947).

*The Theorem of Longuet-Higgins.* Longuet-Higgins (1950a) generalized the result, showing by the LCAO method that, when an *essential* double bond<sup>2</sup> in any chain or even-ring hydrocarbon is twisted by  $90^\circ$ , the  $f^2$  and *fg* configuration energies coincide. The ground state is necessarily a triplet, and  $^1A$  and  $^1B$  must be low and close together.

A molecule excited to the  $^1B$  state may then during its excited lifetime twist in an essential double bond to the minimum energy for that state, near  $90^\circ$ . Thence it may return to the ground state either at  $0^\circ$  or at  $180^\circ$  with about equal probability. Trans-substituted ethylenes will be partly converted to cis after excitation, and vice versa. No fluorescence seems to have been observed in ethylene, which may mean that the twisting motion of the hydrogens has produced internal quenching. But the fluorescence is quite strong in the diphenyl polyenes, where the twisting motion of the heavier rings should be about a hundred times slower.

This indicates that in the latter compounds the quenching time due to twisting is probably between  $10^{-9}$  sec, the fluorescence lifetime, and  $10^{-7}$  sec. Any molecules that reach the longer-lived phosphorescent state will therefore probably be twisted to  $90^\circ$  before they can radiate. It would be interesting to know how long they can remain in the  $90^\circ$  configuration, where this triplet state is the lowest.

As Fig. 2-9 shows, thermal twisting in the  $0^\circ$  configuration will produce only second-order changes in the frequency of the first transition. If the equilibrium configuration is twisted, as by steric hindrance or some other constraint, not only will the frequency be shifted to the red, but thermal twisting will now produce first-order changes. This is presumably the explanation of the red shift of the spectrum from cis-butene to cyclohexene, where an equilibrium twist of about  $20^\circ$  is to be expected, and of the unusually long absorption "tail" on the cyclohexene spectrum, extending into the quartz ultraviolet (Platt *et al.*, 1949). A similar tail extends into the visible region for cyclooctatetraene, making the color of the compound yellow; here a puckered configuration is favored, with each ethylene twisted about  $40^\circ$  (American Petroleum Institute, 1947, 1948).<sup>3</sup>

<sup>2</sup> One that remains a double bond in every principal or nonionic resonance structure, e.g., the center bond in stilbene but not the center bond in biphenyl.

<sup>3</sup> The great width of individual vibration bands in the long-wave-length polyene transitions at room temperatures, as compared with the bands of condensed-ring systems, may be evidence of thermal twisting, with consequent variations in the excited-state energy. It seems to be a general rule that the sharpest bands in solutions are those of rigid planar systems, with maximum resistance to twist. Substitution of methyl or alkyl groups, which have free rotation but little conjugation, broadens the bands. The vibrational structure frequently disappears entirely with more

These observations then offer some support to the theoretical picture given in Fig. 2-9.

### EFFECT OF SUBSTITUTION

*Saturated Hetero Substitution.* The effects of substitution on a pure hydrocarbon conjugated system depend on the nature of the substituent. A summary of the effects was given by Platt (1951a).

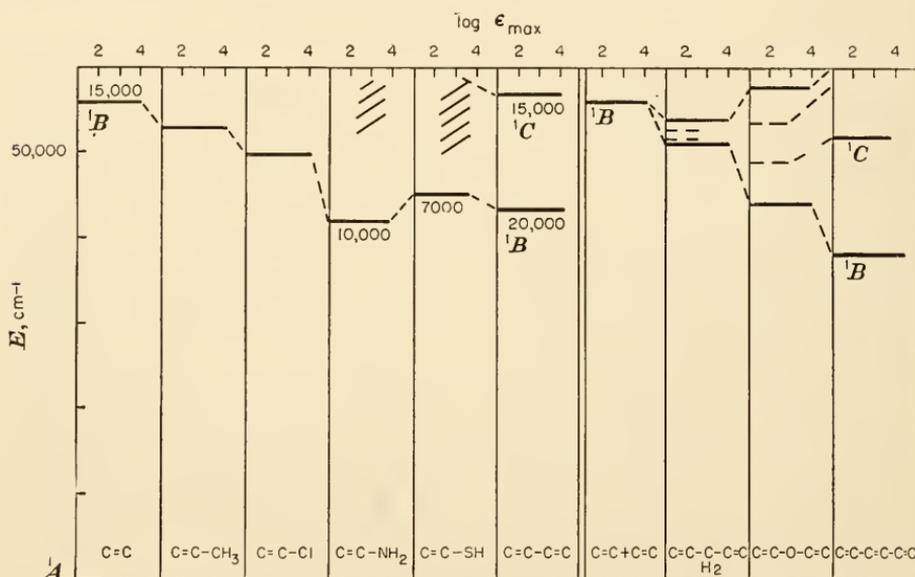


FIG. 2-10. Ethylene substitution by saturated auxochromes (variconjugate sequences).

Formally saturated substituents—"auxochromes"—added to the conjugated system produce shifts to longer wave lengths and general increases in intensity, with especially large relative increases in forbidden transitions if symmetry is destroyed by the substitution. An exception is fluorine, which produces shifts to shorter wave lengths. The magnitude of these effects follows the approximate sequence F, CH<sub>3</sub>, Cl, Br, OH, I,

conjugated substituents that can twist, such as nitro, amine, acid, or aldehyde groups. Rigid heterocyclics often have sharp structure in nonpolar solvents but lose it in polar solvents, possibly because loosely coupled solvent molecules are free to twist (and to execute other motions) and so to vary thermally the energy of the excited state of the whole system. See Merkel and Wiegand (1947).

Kasha (personal communication) has suggested that the well-known loss of structure in the higher transitions of all conjugated systems has a different and more general explanation, namely, the great speed of the higher radiationless transitions. This would be a sort of "internal predissociation" rather than the external or normal predissociation traditionally invoked to account for diffuseness in spectra. If these transitions are as fast as  $0.3 \times 10^{-13}$  sec (three C—H vibration periods), they will produce an uncertainty of  $3 \times 10^{13}$  cycles per second or a diffuseness of 100 Å near 3000 Å, comparable to that shown for β-carotene in Fig. 2-1. In this connection, see especially Lewis and Calvin (1939).

$\text{NH}_2$ ,  $\text{SH}$ . The effects of the last two are so large that they are almost equivalent to the addition of two more  $\pi$ -electrons to the conjugated system, i.e., to a vinyl substitution; and frequencies and intensities may be estimated from those of the vinylog of the original hydrocarbon, as seen in Fig. 2-10.

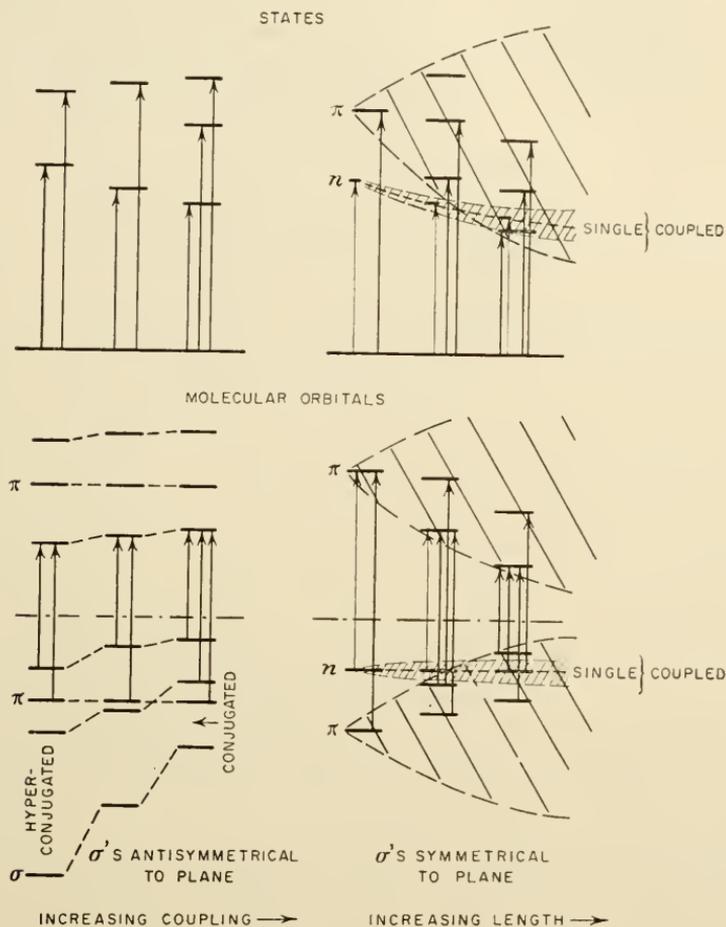


Fig. 2-11. Behavior of hydrocarbon orbitals and states with substitution by saturated auxochromes, and with replacement by conjugated hetero atoms.

Any substituent, even another conjugated system, which is separated from the original conjugated system by a methylene group, or especially by a longer alkyl chain, is insulated from the original system and has scarcely more effect on its spectrum than if it were in another molecule. The absorptions of two conjugated systems which are in the same molecule but which are separated in this way are simply additive.

Auxochromes add no additional bands to the long-wave-length spectra of a conjugated system; at least, none has ever been identified.

The spectra of vitamin A and the carotenes (Fig. 2-1) are obviously standard examples of alkyl-substituted all-trans polyenes.

*Conjugated Hetero Replacement.* The situation is different if we replace some of the carbon atoms in the conjugated system by conjugated hetero atoms,  $\equiv\text{N}$ ,  $=\text{N}$ , or  $=\text{O}$ . These atoms have nonbonding electrons or  $n$ -electrons in high  $n$ -orbitals, as shown in Fig. 2-11. These orbitals may even lie above the highest  $\pi$ -orbitals, or  $f$ -orbitals, in small conjugated systems, although the  $f$  shell in a large enough conjugated system will rise above the  $n$ -energy, which changes little with the size of the system.

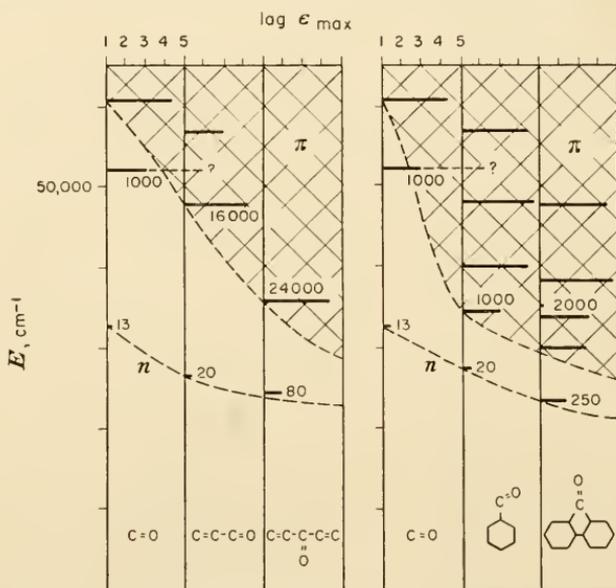


FIG. 2-12. Change of  $n$ - $g$  ( $n$ ) and  $f$ - $g$  ( $\pi$ ) transition frequencies with increasing size of a conjugated system. Figures in columns give extinction values. (From Platt, 1951a.)

The longest-wave-length transition in a small system will then be of  $n$ - $g$  type. With increasing size of the conjugated system the  $g$ -orbital will move, but not the  $n$ , so that the transition frequency will move to the red only about half as fast as the  $f$ - $g$  transition, and will be concealed by the latter in large systems, as shown in Figs. 2-11 and 12. This is one of the main identifying properties of  $n$ - $g$  transitions.

The symbol  $\dots n^2$  must be added to the  $\pi$ -configuration symbols in such molecules, as shown in Fig. 2-7 and Table 2-1. The ground state becomes  $\dots d^2e^2f^2n^2$ ; the  $f$ - $g$  excited configuration,  $\dots d^2e^2fn^2g$ ; etc., each with the same states as before. But we must now add the configuration from  $n$ - $g$  excitation,  $\dots d^2e^2f^2ng$ . This gives singlet and triplet states that we shall call  ${}^1U$  and  ${}^3U$  when the transition from ground is forbidden and  ${}^1W$  and  ${}^3W$  when it is allowed. Two of these states are indicated in Fig. 2-7, and some typical absorption intensities

and lifetimes are shown in Fig. 2-8. The  $n$ - $g$  transitions are weak, with  $\epsilon_{\max}$  between 10 (forbidden) and 1000 (allowed). In the allowed transitions the polarization is predicted to be perpendicular to the molecular plane. The general weakness of the transitions is due to the relatively small overlap between the localized  $n$ -orbitals and the spread-out  $g$ -orbitals.

The result of this weakness, as shown in Fig. 2-7, is a long time constant for fluorescence when the first excited singlet state is  ${}^1U$ , so that the radiationless transition to the lowest triplet is more probable than radiation to ground. At room temperatures the energy goes into the triplet state, which is quenched, and no luminescence is observed. At low temperatures, phosphorescence only is seen. For molecules large enough so that  ${}^1B$  comes below  ${}^1U$ , the behavior reverts to the normal hydrocarbon pattern (Kasha, 1950). [It is also possible for  ${}^1U$  to be the lowest singlet and  ${}^3B$  the lowest triplet. The fluorescence behavior in such cases can be predicted from Figs. 2-7 and 8. These cases are important in the light of Reid's recent demonstration (1953) that the  ${}^1U$ - ${}^3U$  and  ${}^1W$ - ${}^3W$  separations are theoretically and experimentally very small, of the order of  $100 \text{ cm}^{-1}$ .]

The  ${}^1U$  and  ${}^1W$  states have still another identifying characteristic. This is their behavior in polar and acid solvents, which lower the energy of the exposed  $n$ -orbitals, producing strong *blue* shifts of the  $n$ -transitions, just opposite to the shifts of the  $\pi$ -transitions, which move to the red with increasing refractive index (McConnell, 1952). The  $n$ - $g$  transitions are also distinguished by their narrow "atomic-like" vibrational structure in vapor phase, presumably indicating a very small coupling between the  $n$ -electrons and the molecular vibrations, and, conversely, by their almost complete absence of structure in solution, indicating their strong interaction with the solvent molecules (Kasha, 1950).

Where there are two or more conjugated hetero atoms in the same system, their  $n$ -orbitals interact, producing red shifts of the  $n$ - $g$  transitions (Platt, 1951a) and frequently changing the lowest transition from a  ${}^1U$  type to a  ${}^1W$  type, as in going from pyridine to the diazines (Halverson and Hirt, 1951).

The sequence of the highest  $n$ -orbital energies for different hetero groups is shown in Table 2-2, as observed and estimated from the tabulated positions of typical  ${}^1A$ - ${}^1U$  and  ${}^1A$ - ${}^1W$  transitions in a number of compounds (McConnell, 1952).

*Hetero Shifts in Odd-atom Systems.* In certain cases, hetero replacement of carbon produces large shifts in the  $\pi$ - $\pi$  transitions. Kuhn (1950) and Dewar (1950) accounted for a large class of such cases, where symmetrical anilinium ions have a central CH replaced by N. Such an aza substitution on Michler's Hydrol Blue, shown in Figs. 2-2, 6, and 13, converts it to Bindschedeler's Green, with a shift of the  ${}^1A$ - ${}^1B$  absorption

from 6100 to 7100 Å. (These systems are too large for the weak  $n-g$  transitions introduced by the aza nitrogen to be seen.)

Kuhn showed that this shift of the  $\pi$ -transitions could be understood by considering the symmetries of the orbitals, as sketched in Fig. 2-13. The aza substitution attracts electrons. By perturbation theory, this

TABLE 2-2. POSITIONS OF  $n-g$  TRANSITIONS IN DIFFERENT SYSTEMS<sup>a</sup>

Group	Wave length, Å			
	Alone (alkyl-substituted)	On vinyl	Group in phenyl ring <sup>b</sup>	Group on phenyl ring
—CONH—	2000	(2400?)	x	(2700?)
—COOH	2100	2500	x	(2900?)
/  C=N—	(2100?)	(2500?)	2900	(2900?)
\  N=C—C=N—	.....	(2500?)	3400 ( <sup>1</sup> W?)	
—C≡N	.....	(2500?)	x	(2800?)
—NO <sub>2</sub>	2700	2800	x	3300
—ONO <sub>2</sub>	2700	.....	x	
/  C=O	2800	3200	x	3300
\	(1900- <sup>1</sup> W?)			
/  C=S	3300	.....	x	
\				
/  N—N=O	3400	.....	x	
\				
—N=N—	3700?	.....	3400 ( <sup>1</sup> W?)	4040
—O—N=O	3700	.....	x	
—CO—CO—	4600	.....	4500 ( <i>p</i> -Quin.)	
	2800	.....		
—N=O	6800	.....	x	7700

<sup>a</sup> Transitions probably <sup>1</sup>A-<sup>1</sup>U except where <sup>1</sup>A-<sup>1</sup>W is indicated. Parentheses indicate estimates or hidden and unidentified transitions; question marks indicate doubtful assignments of observed bands. Wave lengths approximate (from Platt, 1951a; Braude, 1945; Braude *et al.*, 1947; Barany *et al.*, 1949; McConnell, 1952).

<sup>b</sup> The symbol x indicates that the group cannot go into a phenyl ring.

lowers the energy of orbitals whose wave functions and electron densities are large at the aza nitrogen, and it leaves unchanged the energy of those whose wave functions are zero or small there. Since the aza atom is at the center of a symmetrical molecule, all even orbitals will be lowered, but odd ones will be unchanged, as shown by the short arrows in the center of the figure. In these particular 16-electron systems, the  $f$ -level is odd and unchanged and the  $g$ -level is even and lowered, producing the red shift of 1000 Å observed.

Kuhn found a number of other symmetrical ions with a reversed arrangement, the *f*-level being even and the *g*-level odd. In these the shift on central aza substitution was consequently in the opposite direction, a blue shift, and of about the same amount, 1000 Å, just as expected.

For more complicated molecules with a central atom, the oddness or evenness of the *f*-level may usually be found easily by Longuet-Higgins'

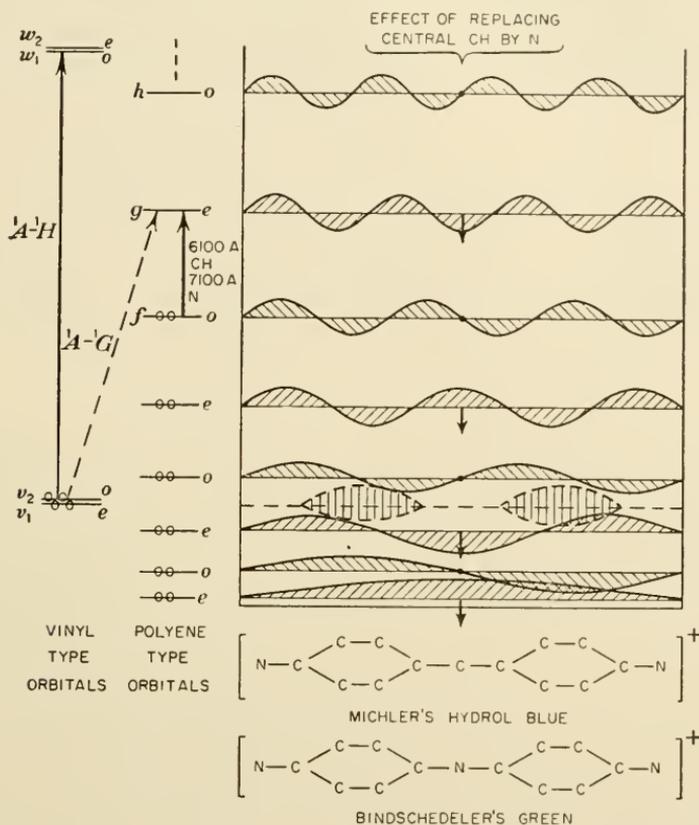


FIG. 2-13. Orbitals and transitions of symmetric phenylpolyene ions, and effect of aza substitution (Adapted from Kuhn, 1950.)

very simple method (LCAO) for "zero-energy orbitals" (1950b), provided that no odd-membered ring systems are present. Dewar (1950) has applied this method to a prediction of substitution effects on spectra of odd-atom dye systems for any position of substitution.

*The Coulson-Rushbrooke Theorem.* The shifts of the  $\pi$ -transitions with aza substitution in most of the stable neutral even-ring hydrocarbons are much smaller than in the ions discussed by Kuhn. This fact has its explanation in the Coulson-Rushbrooke LCAO theorem (1940), which states that any hydrocarbon that has no odd-membered rings and no zero-energy orbitals must have the electron density the same in the

$g$ -orbital as in the  $f$ -orbital at every atom. Aza substitution, or any other substitution, at any position therefore lowers both the  $f$ - and  $g$ -energies by equal amounts, and the transition frequency remains constant in first approximation. Actually a blue shift of 100 or 200 Å is characteristic of aza substitution in these molecules and seems to be independent of the position of substitution. The shifts in Kuhn's ions, on the other hand, should be strongly sensitive to the position of substitution.

The fact that, for stable neutral even-ring hydrocarbons, the fluorescent and phosphorescent states will have electron densities approximately unchanged from those in the ground state means that their photochemistry will be fundamentally different from the photochemistry of odd-ring molecules or molecules with "zero-energy orbitals," such as those of Fig. 2-13, where optical excitation produces large electron transfers from point to point in the system. It would seem that photoexcitation where the electron density is unchanged would be especially likely to lead to simple intramolecular rearrangement, and that chemical reactions would be favored in cases where the electron density changes.

#### RING-CHAIN SYSTEMS

Figure 2-13 also illustrates the resemblance between polyenes and phenyl chain systems, including phenyl polyenes, diphenyl polyenes, and  $p$ -polyphenyls (Platt, 1951a). For every ring in such systems there is one filled orbital  $v$  and one empty orbital  $w$  of a special type. They are called "vinyl type" in Fig. 2-13 because they have about the same energy as the ethylene  $f$ - and  $g$ -orbitals, respectively, and this energy is almost independent of the number of rings or of the lengths of the polyene parts of the chain.

The remaining orbitals are independent of these orbitals and are called "polyene type," since they are just the same in number as the orbitals of a simple polyene of the same length, and each orbital resembles closely its polyene counterpart in its symmetry and number of nodes, electron distribution, and energy. In computing the length of the equivalent polyene, each phenyl counts as four atoms. The orbital similarity accounts for the similarity of the long-wave-length spectra of the polyenes to those of phenyl chains, which was one of the earliest important results of spectral comparisons.

Substitution of a vinyl group on the side of a polyene to make a phenyl ring then does not much affect the long-wave-length spectrum of the polyene. Such spectral stability is frequently observed in large conjugated systems, provided the smaller systems that are added are at the side of the large one and not at the end.

Transitions among the polyene orbitals,  $f$ - $g$ ,  $e$ - $g$ , etc., will be polarized approximately along the length of the molecule if it is in the all-trans configuration or is as extended as possible. Transitions between the

vinyl orbitals,  $v-w$ ,  ${}^1A-{}^1H$ , etc., will be polarized in approximately the same direction—along the 1,4 axes of the rings.

Transitions between the two groups of orbitals,  $v-g$ ,  ${}^1A-{}^1G$ , etc., will be polarized in the plane of the rings but perpendicular to the 1,4 axes. They will be weak, since the  $v$ -orbital is localized, whereas the polyene orbitals are spread out. They will move to the red only about half as fast with increasing chain length as the  $f-g$  orbitals. This is the reason that the first transition in benzene and styrene, which is presumed to be of the  $v-g$  type,<sup>4</sup> becomes hidden by the stronger  $f-g$  transition in the

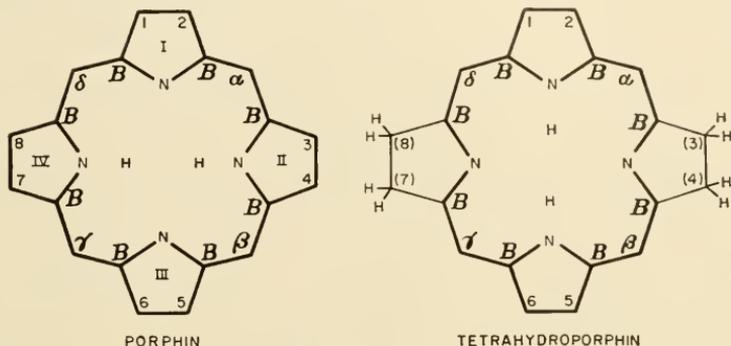


FIG. 2-14. Structure and numbering of positions of porphyrin and tetrahydroporphyrin.

longer molecules phenyl butadiene and biphenyl. No  $v-g$  transitions have been identified in longer ring-chain systems; low-temperature studies are needed.

Some strong absorptions that did not move much with increasing chain length were found in phenyl polyenes by Smakula (1934) near 2000 Å. These may be the  $v-w$  transitions,  ${}^1A-{}^1H$ .

The conclusions reached here about the classification and sequence of transitions in ring chains do not depend on the location of the rings along the chain. Thus the  $\pi$ -electron transitions in quinone are almost identical with those in styrene, which has the same chain length; quinone, of course, has additional weak  $n-g$  transitions at long wave lengths, from the oxygen nonbonding electrons.

Analogues of phenyl chains, in which the benzene rings are replaced by thiophene or pyrrole rings, will have similar spectra, except where forbidden transitions in a symmetrical phenyl compound may be enhanced by the loss of symmetry [see Sease and Zechmeister (1947) on polythienyls].

Naphthyl chain systems seem not to have been examined theoretically, but it seems likely that the result will be similar: When the chain becomes

<sup>4</sup> It would not be the first transition except that it is pushed to long wave lengths (and made weak, in benzene) by electron interaction (Platt, 1950, 1951a) between the configurations we are here calling  $fg$  and  $vw$  (which would be degenerate in benzene). A similar interaction seems to occur in porphyrin, described later in this chapter.

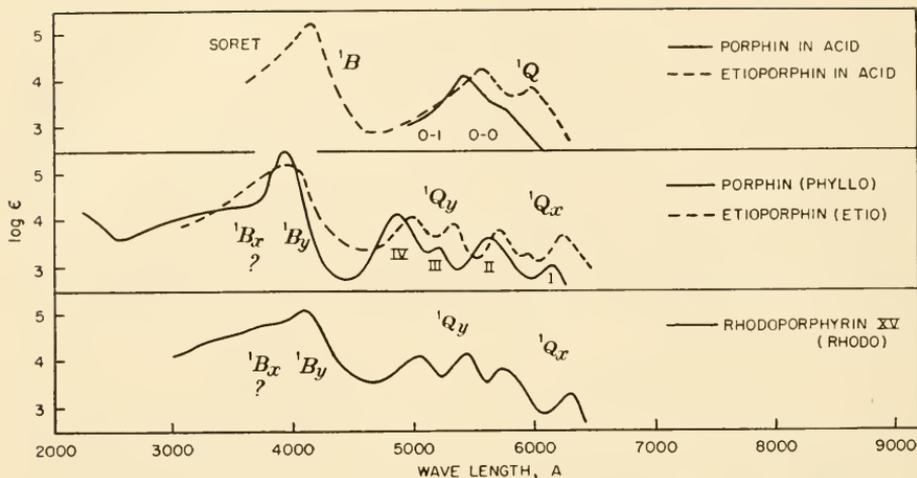


FIG. 2-15. Absorption of porphyrins. (Etioporphyrin spectra from Erdman and Corwin, 1946; others from Stern and Wenderlein, 1935, and Pruckner and Stern, 1936.)

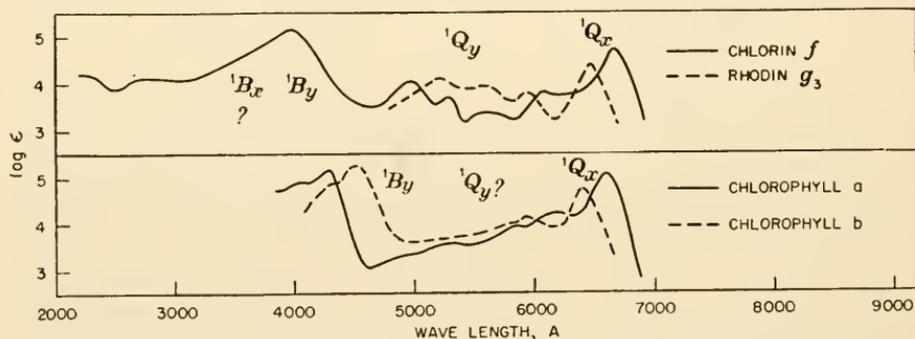


FIG. 2-16. Absorption of chlorins. (Chlorin from Stern and Wenderlein, 1935; rhodin from Pruckner and Stern, 1936; chlorophylls adapted from Zscheile and Comar, 1941.)

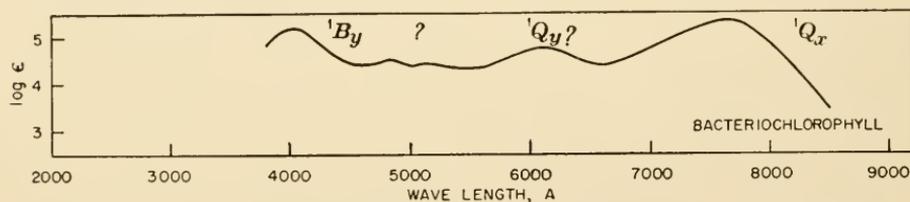


FIG. 2-17. Absorption of bacteriochlorins. (Adapted from French, 1937.)

longer than the naphthyl group itself, the long-wave-length spectra will begin to have predominantly polyene character.

### PORPHYRINS

After the polyenes, the porphyrins open up new vistas of sophistication and interest. These compounds comprise

1. The true porphyrins, which are derivatives of *porphin* (Fig. 2-14);

2. Chlorins (including rhodins), which are derivatives of *dihydroporphin*; and

3. Bacteriochlorins, which are derivatives of *tetrahydroporphin* (Fig. 2-14) (Rabinowitch, 1944; Aronoff, 1950).

Typical spectra of these compounds are shown in Figs. 2-15, 16, and 17.

### PORPHIN

*One-electron Orbitals.* The orbitals and transition energies were computed for porphin and tetrahydroporphin by Longuet-Higgins *et al.* (1950) using the LCAO molecular-orbital approximation.<sup>5</sup> The highest filled and lowest empty orbitals are shown in Fig. 2-18, and the centers of

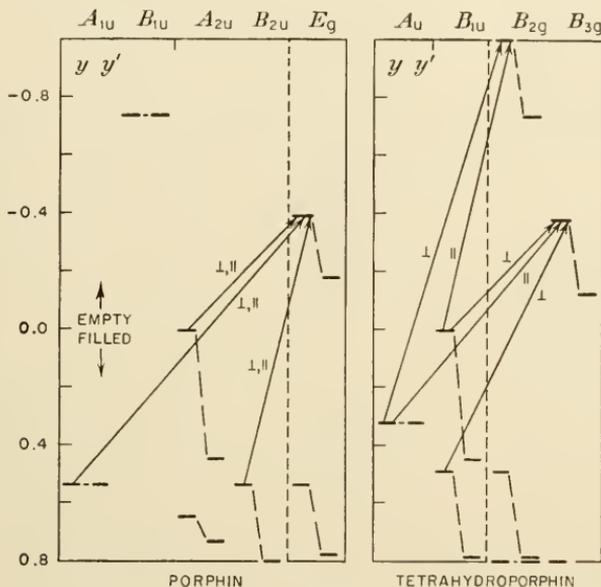
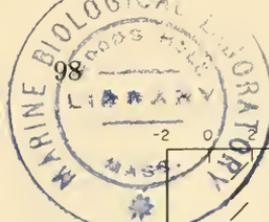


FIG. 2-18. Orbital energies according to symmetry classes, and lowest allowed transitions in porphin and tetrahydroporphin. The double symbol  $\perp, \parallel$  indicates double degeneracy. The single symbols indicate polarization with respect to the longest axis of the conjugated system in tetrahydroporphin.

gravity of the first excited configurations are shown after them in Fig. 2-19 for comparison with the observed excited singlet states. The orbitals in the columns marked  $y$  were computed assuming the central nitrogen atoms to be equivalent to carbon atoms. Those marked  $y'$  were computed with a higher electron affinity at the nitrogen positions, all nitrogen atoms still being assumed equivalent to each other. The predicted configuration energies from either set of orbitals fit the observed singlet levels about as well as predictions fit in other molecules when made by the same methods (Platt, 1950). The symbols at the top of Fig. 2-18

<sup>5</sup> Including consideration of the overlap integral.



RADIATION BIOLOGY

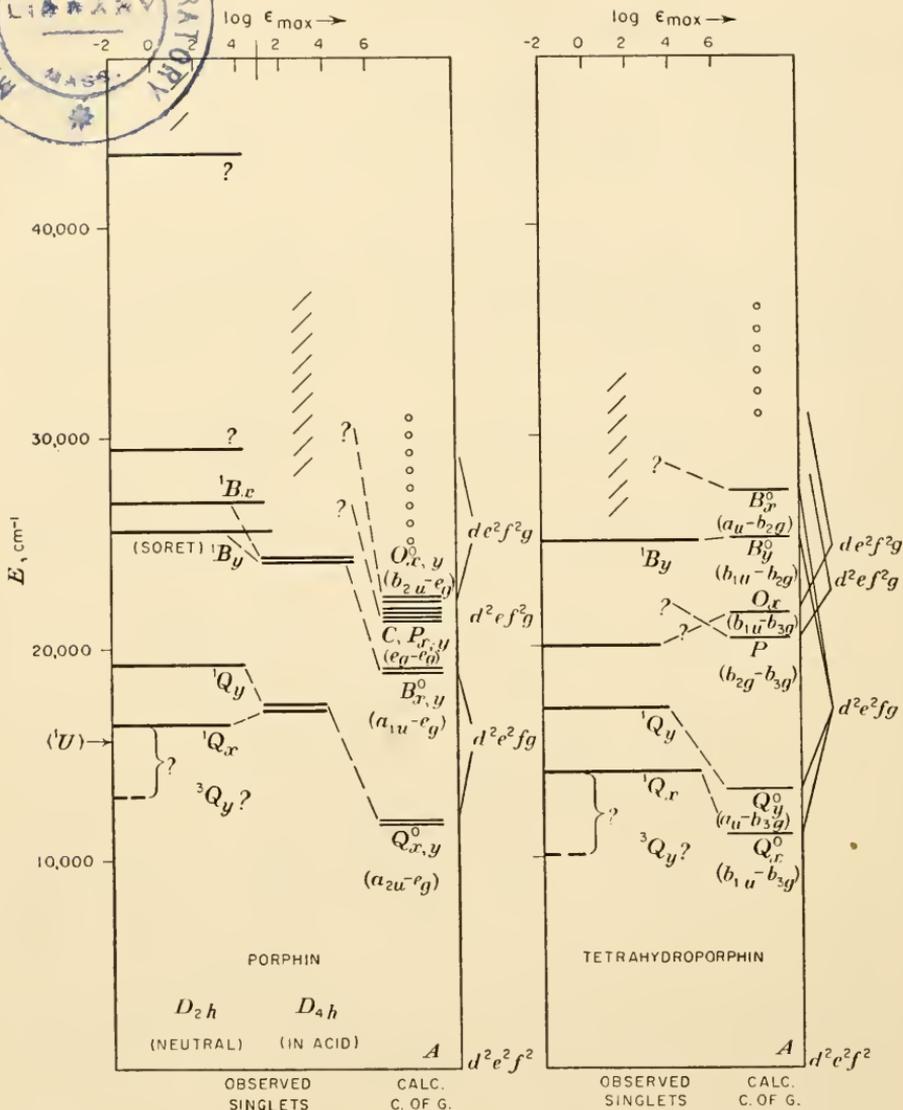


FIG. 2-19. Comparison of calculated with observed energy states for porphyrin and tetrahydroporphyrin. Calculations corrected for nitrogen electron attraction (half that assumed by Longuet-Higgins *et al.*, 1950). In "observed" columns, vibrational structure is omitted for simplicity. Length of horizontal lines indicates approximate intensity of transition from ground state. Double lines indicate double degeneracy. Slant lines indicate unknown region. Dots indicate numerous additional calculated levels. Arrow at left of porphyrin diagram shows relation of hypothetical  ${}^1U$  state to  ${}^1Q_z$  in chlorophyll.

refer to the symmetry of the wave functions in standard symmetry notation; in the calculations porphin was assumed to have  $D_{4h}$ , or square, symmetry.

*Angular Momentum and Vector Addition.* The fundamental difference between porphyrins and the polyenes stems from the fact that the conjugated system of porphyrins is not linear but has a two-dimensional extension in its own plane. As a result, some orbitals of porphin such as the lowest unfilled one (in the  $E_g$  column of Fig. 2-18), which we again call the  $g$ -orbital, are doubly degenerate. That is, they consist of two orbitals, one pointed in the  $x$  direction of the square, the other in the  $y$  direction, the two components being of equal energy because these directions are physically indistinguishable if and when porphin is square.

Or we may equally well think of the electron as switching rapidly from one of these components to the other and so traveling clockwise (one component) or counterclockwise (the other component) around the ring. This degeneracy in the  $g$ -orbital makes each of the long-wave-length transitions of porphin also doubly degenerate, with two components polarized in mutually perpendicular directions (Fig. 2-19).

In a two-dimensional conjugated system it also often happens that other pairs of orbitals that are not strictly degenerate, like the  $E_{2g}$ , are nevertheless almost degenerate, as, for instance, the highest filled pair,  $A_{1u}$  and  $A_{2u}$ , in the  $y'$  columns of porphin. This suggests a modification of the LCAO results, as follows: To a certain approximation we may treat such a pair of orbitals in the same way that we treated the  $E_g$  orbital, i.e., as though they were components of a doubly degenerate  $f$ -orbital, with the electron in one component moving clockwise around the ring, in the other counterclockwise (Platt, 1949, 1950).

This combined  $f$ -orbital may then be thought of as having an angular momentum, in this case with a value of four atomic units; i.e., the  $A_{1u}$  and  $A_{2u}$  components out of which we made this orbital are each crossed through the center by four nodal lines. They are both odd functions, which change sign on inversion in the center of symmetry. The  $g$ -orbital, of  $E_g$  type, is even and is crossed by five nodal lines through the center, so that it has an effective angular momentum of 5 units.

On exciting an electron of angular momentum 4 to angular momentum 5, the two momenta may be either in the same direction, and add, or in the opposite direction, and subtract. The whole molecule, which began in its ground state  $^1A$  with total angular momentum 0, may then change its angular momentum to a value of 1 unit ( $5 - 4$ ) clockwise or counterclockwise, or of 9 units ( $5 + 4$ ) clockwise or counterclockwise. These two values of momentum will give two degenerate singlet states and two degenerate triplet states for the first excited configuration, . . .  $d^2e^2fg$ . We call these states  $^1B^\circ$ ,  $^1Q^\circ$  and  $^3B^\circ$ ,  $^3Q^\circ$ , where  $B$  refers to 1 unit of angular momentum and  $Q$  to 9. Electron interaction will

push the  $B$  and  $Q$  states apart, with the  $Q$  lying lower (Hund rule); triplets of each type will again lie below singlets of the same type.

The important prediction that this trick of adding angular-momentum vectors makes possible is that transitions from the ground state to the low  $Q$  states will be comparatively weak because large changes of angular momentum are forbidden, whereas the transition to the higher  $B$  states will be strong and highly allowed. This is precisely the difference between the porphyrin absorptions to the two lowest excited states, as shown in Fig. 2-19. In the visible bands of porphyrin, near  $18,000\text{ cm}^{-1}$ , the observed molar extinction  $\epsilon_{\text{max}}$  is about 10,000; in the violet or "Soret" bands near  $24,000\text{ cm}^{-1}$ , it is about 200,000. This is a general result: Comparatively weak long-wave-length transitions in  $\pi$ -electron spectra are peculiar to molecules extended in two dimensions, especially symmetrical ones.

Here the symbol  $Q$  has been given to the low state of high momentum, instead of the symbol  $L$  that was used for the same kind of state in benzene and the condensed-ring systems (Platt, 1949), because the properties of the  $Q$  state are very different, for instance, in its behavior with chemical substitution, as will be seen later.

The prediction of weakness of the first transition by the vector model is a result not easily obtained by the usual (one-electron) method, which simply predicts that the first two transitions of porphyrin will be allowed. Configuration interaction must be considered in such calculations before any substantial difference is found in the predicted intensities of the first two transitions. Simpson (1949) first made this prediction of weakness for porphyrin by applying vector addition to a free-electron model, but he oversimplified the problem unnecessarily by restricting his electrons to a closed 18-atom loop, leaving out of consideration six other atoms in the porphyrin conjugated system. When some of these atoms are actually missing from the conjugated system, so that it is more similar to Simpson's model, the first transition loses its weakness, as will be seen later, and no longer agrees with Simpson's prediction.

Summarizing, we identify the visible bands of square ( $D_{4h}$ ) porphyrin from these theoretical considerations as the  $e^2f^2\ ^1A - e^2fg\ ^1Q^o$  transition, degenerate and almost forbidden, and the Soret band near 4000 Å as the  $e^2f^2\ ^1A - e^2fg\ ^1B^o$  transition, degenerate and strongly allowed.

#### STRUCTURE OF THE VISIBLE BANDS

*Configuration of Porphyrin.* In neutral porphyrin the only parts that *must* violate the  $D_{4h}$  symmetry are the two central hydrogens. Discussion raged for years about whether they were normally on opposite nitrogen atoms or on adjacent nitrogen atoms or served as bridges between the nitrogen atoms; or whether all these species were present in an equilibrium mixture, with rapid transformation from one to the other. This problem

was settled by Erdman and Corwin (1946), who showed that replacing one of these protons by a methyl group produced little change in the spectrum. Such a N-methyl group would be expected to be rather tightly bound to a single nitrogen and should not undergo very rapid shifts of position to another nitrogen. Also, in the presence of the bulky methyl group, the remaining proton would probably prefer the opposed position for steric reasons. The adjacent configuration, the bridge configuration, and the rapid-transformation hypothesis are therefore unnecessary in accounting for the spectrum. It suffices to assume that the protons are bound by normal covalent bonds to opposed nitrogen atoms.

There are three ways of modifying porphin so that it can have strict  $D_{4h}$  symmetry: (1) we may remove the central hydrogens, as in the disodium salt; (2) we may add two additional hydrogen atoms, as in the dihydrochloride or in concentrated acid solution; or (3) we may replace the central hydrogen atoms by a single central atom, as in the copper or zinc complexes. Any of these changes produces a great simplification in the visible spectrum, as seen for the dihydrochloride in Fig. 2-15 (top), and, except for small wave-length shifts, all three changes produce very similar absorption curves!

Porphin itself in acid has an  $A-Q$  transition with only a single sharp peak, and its  $A-B$  transition becomes extremely sharp. *This* must be the spectrum which belongs to the square  $D_{4h}$  compound we treated theoretically, with strict degeneracy.

*The Interpretation of the Visible Bands.* The observed single  ${}^1A-{}^1Q$  peak in acid can be interpreted as a 0-1 vibrational band, normally the second peak in an absorption-band system. The first, or 0-0 band, is completely absent, as it would be in a strictly forbidden transition, and it appears only when the system is slightly perturbed, as by alkyl substitution [etioporphyrin in acid (Fig. 2-15)]. The one observed  ${}^1A-{}^1B$  peak in acid is presumably the 0-0 band, for it does not acquire a longer-wave-length companion with alkyl substitution.

If this is the  $D_{4h}$  spectrum, the complexities of the free-base porphin spectrum are evidently due to the change to  $D_{2h}$ , or rectangular, symmetry, with removal of the degeneracy, when two protons only are present in the center. A change of symmetry from external substitution on the ring gives less dramatic spectral changes than when the central symmetry is altered.

The visible spectrum of neutral  $D_{2h}$  porphin looks like a superposition of two of the  $D_{4h}$  spectra, shifted 800 Å apart. This separation is what would be expected when the degeneracy of the upper  ${}^1Q$  state is removed. It therefore seems reasonable to assign the four visible bands I, II, III, IV (numbered from the red end) as follows:

Bands I and II: 0-0 and 0-1 vibrations of  ${}^1A-{}^1Q_x^0$ ,

Bands III and IV: 0-0 and 0-1 vibrations of  ${}^1A-{}^1Q_y^0$ ,

where  $Q_x$  and  $Q_y$  are polarized in mutually perpendicular directions, whose relations to the  $H-H$  axis of the central protons has yet to be determined.

### EFFECT OF SUBSTITUTIONS

The effect of substituents on forbidden transitions is easier and more entertaining to compute than the effects on allowed transitions. In the porphyrin visible bands, the intensity changes with substitution are especially interesting. Several "types" of spectra have been distinguished (Stern and Wenderlein, 1936, V; Rabinowitch, 1944) according to the relative intensities of the different band maxima and have been roughly correlated with the presence or absence of certain kinds of substitutions. The spectra are different enough to be used for following reactions, for identification, and for analysis.

*Spectroscopic-moment Vectors.* The theory of intensity changes in the forbidden 2600 Å bands of benzene on substitution was given by Sklar (1942) and Förster (1947). Substituents cannot decrease the native (vibrational) intensity of a forbidden transition, but can only increase it by destroying the symmetry that makes it forbidden and by mixing the excited-state wave function with the wave function of an allowed transition.

Briefly, the result of the theory is that the increase of intensity over the unsubstituted compound is proportional to the square of a vector, the transition-moment integral of Eq. (2-12), or rather that part of it induced by the presence of the substituents. This induced vector is the sum of vector components, one for each substituent. The magnitude of each component, or its "spectroscopic moment," which may be either positive or negative, is a parameter that depends only on the substituent; its direction depends only on the position of substitution. (See Fig. 2-20 for typical vector directions as functions of position of substitution, and Fig. 2-21 for typical vector sums with several substituents. The spectroscopic-moment vector must not be confused with the angular-momentum vectors described earlier.)

This theory was applied to a determination of the moments of 25 benzene substituents from the observed spectral intensities of the 2600 Å bands (Platt, 1951b). It was shown that the moments were roughly proportional to the chemical "directing power" of the substituents, with ortho-para-directing substituents having positive moments, and meta-directing substituents, negative ones.

*Application to Porphyrins.* This theory can be adapted to interpret the changes in the visible bands of the porphyrins. The reasoning is as follows:

Substitution in porphyrins usually seems to strengthen the I and III, or 0-0, bands, of the two electronic components, leaving almost unchanged the II and IV, or 0-1, bands. Therefore the  ${}^1A^{-1}Q^0$  wave functions are

being mixed with the wave functions of nearby allowed transitions that have strong 0-0 absorption peaks. Probably these are the  ${}^1A-{}^1B^0$  transitions. We shall show how the peak intensities can be predicted in bands I and III, which grow from essentially zero values in  $D_{4h}$  porphin.

The available theory of the vector directions of the moments has not been rigorously justified, but it suggests that the directions of positive moments at different positions on porphin might be related as indicated in Fig. 2-20*a* for the two components of  ${}^1A-{}^1Q^0$ . In each diagram the dashed line shows the position of the essential nodal plane of the wave function. The polarization of the transition should be formally perpendicular to this plane. The positions of the central hydrogen atoms and the assignment of the observed bands to these electronic components were not given by the theory. How they were determined we shall see.

Note that in each component, for positive substituents at exactly opposite points on the ring system, the vector directions are parallel. Their moments will add. Such disubstitution at opposite points will produce double the moment and four times the intensity change produced by monosubstitution. This general prediction is more soundly based than the special vector-moment directions in Fig. 2-20*a*, since it must be true for every even-odd transition in centrally symmetric molecules (Platt, 1951*c*). In even-even transitions the moments from opposed disubstitutions must cancel. This will be a useful test in the classification of transitions. In Fig. 2-22 it is seen that both bands I and III grow with increasing alkyl substitution up to octaalkyl porphin (compound 7); there is no evidence of cancellation of moments. Consequently the predicted even-odd character of the visible transitions is confirmed. Disubstitution does not give so much as four times the intensity of monosubstitution, but this was true also in benzene and is presumably due to the deficiencies of the simple first-order theory.

The more detailed predictions of Fig. 2-20*a* require more examination. In principle, if (1) accurate data on enough different porphyrins were available, if (2) the theory were valid to a few per cent, and if (3) the peak molar extinction  $\epsilon_{\max}$  were strictly proportional to the squared transition-moment integral, then we could determine the following quantities for each electronic transition:

1. From different substitutions at a single position, the spectroscopic moment of each substituent;
2. From a single substituent placed at different positions, the value of its moment as a function of position, which is not necessarily constant (three different values being possible, as at positions 2,  $\alpha$ , and 3, plus the central nitrogen atom), and so the values of all moments as functions of position, since their ratios should be constant in a first-order theory;
3. From di- and polysubstitution with one substituent, the exact direction of the vectors at every position (three different directions to be deter-

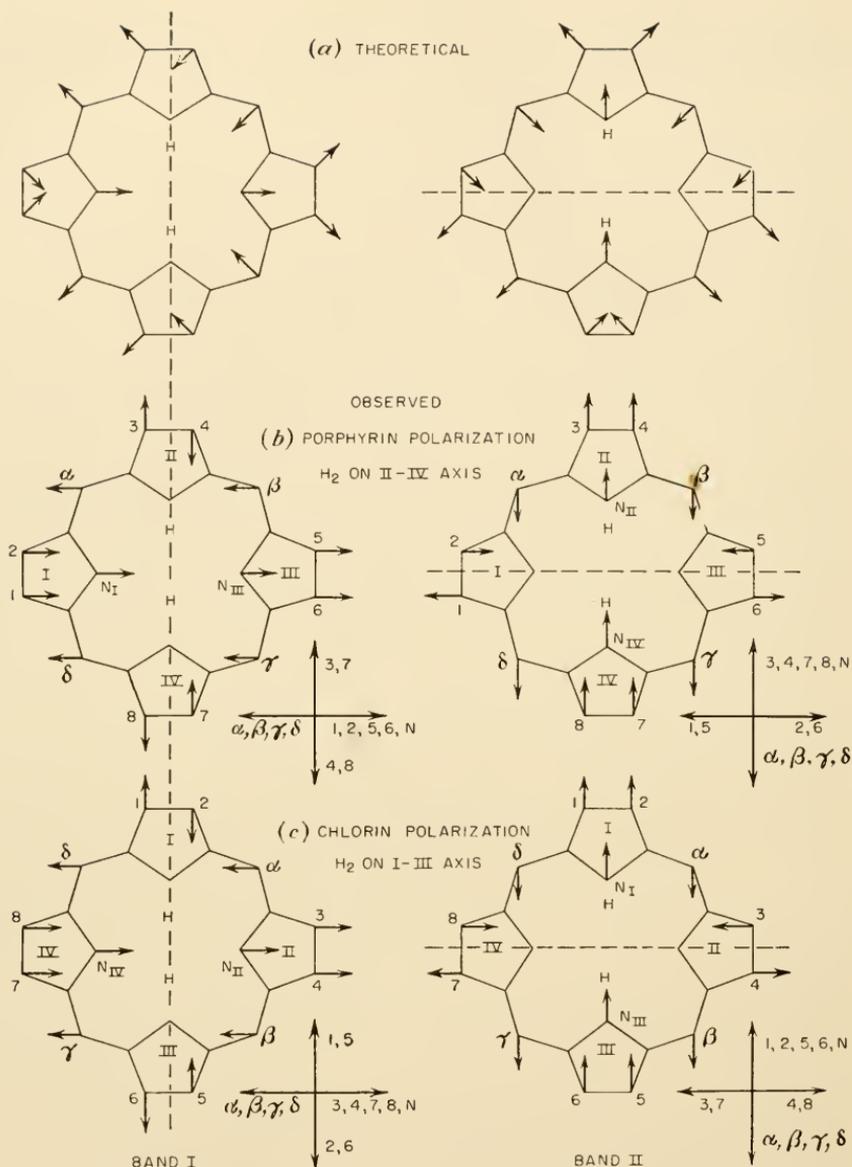


FIG. 2-20. Theoretical and observed vector directions of porphyrin moments at different positions of substitution.

mined, as the central-nitrogen-atom direction in the Erdman-Corwin model is fixed by symmetry); and

4. From all these data, the symmetry of the band I wave function and of the band III wave function with respect to the II-H axis.

The determination of point 4 would not be so straightforward as the others, because the labile central hydrogen atoms may very well be on the I and III rings of porphyrin in one compound and on the II and IV

rings in a slightly different compound. The vector directions will then shift, as in going from Fig. 2-20*b* to *c*, and neighboring vectors that canceled in the one compound may add in the other, as at positions 2 and  $\alpha$  in the diagrams. But even this added complication can be resolved if we stabilize the H-H direction by two very strong opposite substituents and then examine the effect of weaker substituents at all other positions.

In view of the theoretical and experimental limitations of provisos (1), (2), and (3), this program is overambitious. Nevertheless, by examining the data on the first 84 compounds in the comprehensive studies of Stern and Wenderlein (1934, 1935, 1936; Stern, Wenderlein, and Molvig, 1936), as summarized in Fig. 2-22, and by making some simplifying assumptions, a start can be made.

These assumptions are as follows: All alkyls, cycloalkyls, and alkyls bearing an insulated auxochrome on the outer end are treated as equal. Acids and esters are treated as equal. It proves possible to treat hydrogen *addition* to the external rings, with formation of the di- and tetrahydroporphins, also as a perturbation like substitution. The effects on intensity are like those found with strong positive (*o,p*-directing) substituents at each of the hydrogen positions.

The central metal atom in complexes can be treated like a substituent on the central nitrogen atoms.

*Polarizations of Bands I and III.* The first four methyl groups on opposite rings (as 1,2,5,6 substitution) enhance band III strongly but not band I (Fig. 2-22, compound 2). The next four (compound 7) do not enhance band III further but enhance band I to about the same intensity. This proves that the 1,2,5,6 positions are not equivalent to 3,4,7,8 in either band and that band I is polarized perpendicular to band III. [After this chapter had been prepared, Stupp and Kuhn (1952) gave an experimental proof that the corresponding bands in chlorophyll, which we discuss later, are also polarized perpendicular to each other.] Therefore the essential nodal lines of the wave functions in either band cannot pass between the central nitrogen atoms (this would make all four rings equivalent); they must pass through the central nitrogen atoms. This confirms Erdman and Corwin's conclusion that the central hydrogen atoms are not on adjacent nitrogen atoms and are not on opposed N—H—N bridges. It is even possible from this argument and from the intensity data to set an upper limit on the relative abundance of that tautomer which has adjacent hydrogen atoms. Its abundance must be less than 6 per cent under the conditions of Stern and Wenderlein's measurements.

The fact that the total increase of intensity in band I under octaalkyl substitution is almost identical with that in band III shows that the moments of substituents are essentially the same for both transitions. This seems theoretically reasonable.

For band I the data on compounds 2 and 7 show further that the 1- and 2-vectors must cancel and the 3- and 4-vectors add. Examination of the  $\alpha$ -substitution data shows that the  $\alpha$ -vector cancels the 3-vector. These two facts are consistent with the tentative theoretical predictions of Fig. 2-20a if we suppose the essential nodal plane of band I passes between the 1 and 2 positions, or more generally through that pair of opposite rings which has the most or strongest *o,p*-directing substituents. (These are the II and IV rings in that large group of porphyrins which has a strong meta-directing substituent in the 6 position.) It seems chemically reasonable to suppose that the central hydrogen atoms will tend to be on this pair of rings.

A similar, but completely independent, inference from the band III intensities shows that the essential nodal plane of band III passes through that pair of opposite rings which has the least or weakest *o,p*-directing substituents.

Consequently the nodal plane of band I probably coincides with the H-H axis, and the  ${}^1A-{}^1Q_x^o$  bands I and II are probably polarized perpendicular to this axis. The nodal plane of band III is probably perpendicular to the H-H axis, and the  ${}^1A-{}^1Q_y^o$  bands III and IV are probably polarized parallel to this axis.

Hydrogen addition, as in the di- and tetrahydroporphins, is sufficiently different from ordinary substitution to be anomalous. It acts on the intensities like a positive, or *o,p*-directing, moment, but it undoubtedly pushes the central hydrogen atoms away to the other rings [the I and III rings in the usual designations (Fig. 2-20c)]. The intensities can be accounted for if the polarizations are determined *with respect to the H-H axis* according to the rules just given, not if they are determined with respect to the (apparent) strong *o,p*-axis.

The deduction, from this reasoning, that band I will be perpendicular to the H-H axis in tetrahydroporphin agrees with the earlier conclusion of Longuet-Higgins and coworkers (1950), which was based on empirical analogies with condensed-ring spectra and on semiclassical arguments. [It does not agree with Kuhn's predictions (1950), but the amino nitrogen atoms were neglected in his treatment.]

The assignment of the low transition as the transverse  ${}^1A-{}^1Q_x$  implies that the highest transition of the *f-g* group in porphiu (with degeneracy removed) and in tetrahydroporphin must also be transverse and so must be the  ${}^1A-{}^1B_x$ , with the two longitudinal transitions  ${}^1A-{}^1Q_y$  and  ${}^1A-{}^1B_y$  in between. This agrees with the assignments of Longuet-Higgins *et al.* The  ${}^1A-{}^1B_x$  transition in chlorins and bacteriochlorins will be weaker than its longer-wave-length companion, the strong  ${}^1A-{}^1B_y$  Soret band, because much of its intensity has been contributed to the  ${}^1A-{}^1Q_x$  transition, as we shall see.

*Empirical Determination of the Vector Directions.* Returning to the

tetra- and octaalkyl data, we see further that either the 1,2,5,6 moments are less than one-fourth as large as the 3,4,7,8 moments if the position-1 and -2 vector directions of Fig. 2-20a are correct, or these tentative vector directions are in error by at least  $45^\circ$  if the moments at all positions are taken equal. Examination of the meta-directing 6-substituents given in Fig. 2-22 (compounds 9-15) showed that the predicted 3-vector and with it the  $\alpha$ -vector must also be in error by about  $45^\circ$  or by about  $135^\circ$ ; this conclusion did not depend on the equality of different positions. The best agreement with the theoretical predictions is obtained if we choose the  $45^\circ$  error.

Such an error is not unreasonable if we remember that the theoretical directions were derived for a  $D_{4h}$  porphin with all bonds equal. The directions depend on the phase of the wave functions at the substituted atom. In an upper state with effective angular momentum 9, the phase will change by about  $180^\circ$  in one bond distance. Alternations in bond length, plus the change to a strongly  $D_{2h}$  structure, may therefore easily produce changes in moment directions of many degrees.

With the directions so altered, the data can now be fitted taking the moments equal at all external positions. This assumption in turn fixes the vector directions more accurately. Within an uncertainty of about  $20^\circ$ , they are as given in Fig. 2-20b and c. Surprisingly enough, they are almost perpendicular or parallel to the essential nodal plane in every case and may be taken exactly so for convenience in intensity computations.

We may call the polarizations of Fig. 2-20b "porphyrin polarizations" and those of Fig. 2-20c "chlorin polarizations."

*Determination of Moments.* Once the vector directions are established, we can find the values of the moments from the intensity data. The values adopted in Table 2-3 fit the data fairly well. They are given in units such that the square of their vector sum will be numerically equal to the peak molar extinction.

They are uncertain by about 20 per cent. Some of the uncertainties are interdependent; an increase of one value would require lowering or raising another to maintain the fit with the data. A change in the vector directions would also entail adjustments in the values. The fit with the data, as shown in Fig. 2-22, is therefore somewhat better than the accuracy of the individual moments.

The spectroscopic moments of four of these substituents had been determined previously for the benzene 2600 Å bands: alkyl (+6, +7), nitrile (-19), acid (-28), imino (-38) (Platt, 1951b). The moments in porphin are roughly proportional to these, but about two or three times as large. The difference is partly due to a difference in the way the data were smoothed in benzene, and partly due to our application of the theory to  $\epsilon_{\max}$ . A quantity of more theoretical significance would be the contribution  $Q$  of a substituent to the transition-moment integral,

TABLE 2-3. ADOPTED SPECTROSCOPIC MOMENTS FOR PORPHIN SUBSTITUENTS

Substituent	Type of derivative	Spectroscopic moment, (moles/liter) <sup>-1/2</sup>	Code <sup>a</sup>			
H	Hydro (addition)	+90	9			
Me						
—CO—COOH				Glyoxylic acid	70	7
—C=NOH				Oxime	30	3
—C=C—				Vinyl	30	3
—R				Alkyl, cycloalkyl, alkyl acid, etc.	20	2
—H	Unsubstituted	0				
—CO—R	Formyl, acetyl	-30	$\bar{3}$			
—CO—C <sub>6</sub> H <sub>5</sub>	Benzoyl	-40	$\bar{4}$			
—C≡N	Nitrile	-50	$\bar{5}$			
—COOR	Carboxylic acid, ester	-50	$\bar{5}$			
=N—	Imino (replacing CH)	-60	$\bar{6}$			
—C=C—COOR	Acrylic acid, ester	-80	$\bar{8}$			
Mg	Metal, complexing	+90	Mg			
Fe	Metal, complexing	0	Fe			

Isocyclic rings connecting  $\gamma$ -6 positions

—CH <sub>2</sub> —CO—	Like $\gamma$ —CH <sub>3</sub> plus 6—COOH	....	* <sub>a</sub>
—CH(COOCH <sub>3</sub> )—CO—	Like $\gamma$ —CH <sub>3</sub> plus 6—COOH	....	# <sub>a</sub>

<sup>a</sup> Used in summarizing the structure of the compounds in Fig. 2-23.

which can be obtained from the area under the absorption curve for the whole electronic transition. Estimates of this area in a few cases indicate that  $Q$  is about 30 per cent larger in the porphins than in the benzenes. The difference may indicate a greater polarizability of the porphin ring system.

Figure 2-21 shows how the vector moments are added to give the resultant moment for a few compounds. The direction of the resultant is shown with respect to the H-H axis. This should be the direction of polarization of the absorption or emission and may be experimentally tested if oriented crystals of the compounds are available. The directions of polarization of the two electronic components are no longer necessarily perpendicular after substitution has destroyed the symmetry.

*Intensity Formulas.* The fact that the individual moment vectors are at right angles enables us to write simple formulas for the total change of intensity of band I or band III. Let

$$\begin{aligned}
 m_1 + m_5 &= M_1; m_2 + m_6 = M_2; \\
 m_3 + m_7 &= M_3; m_4 + m_8 = M_4; \\
 m_\alpha + m_\beta + m_\gamma + m_\delta &= M_\alpha; m_{N_1} + m_{N_2} = m_{N_2} + m_{N_4} = M_N.
 \end{aligned}$$

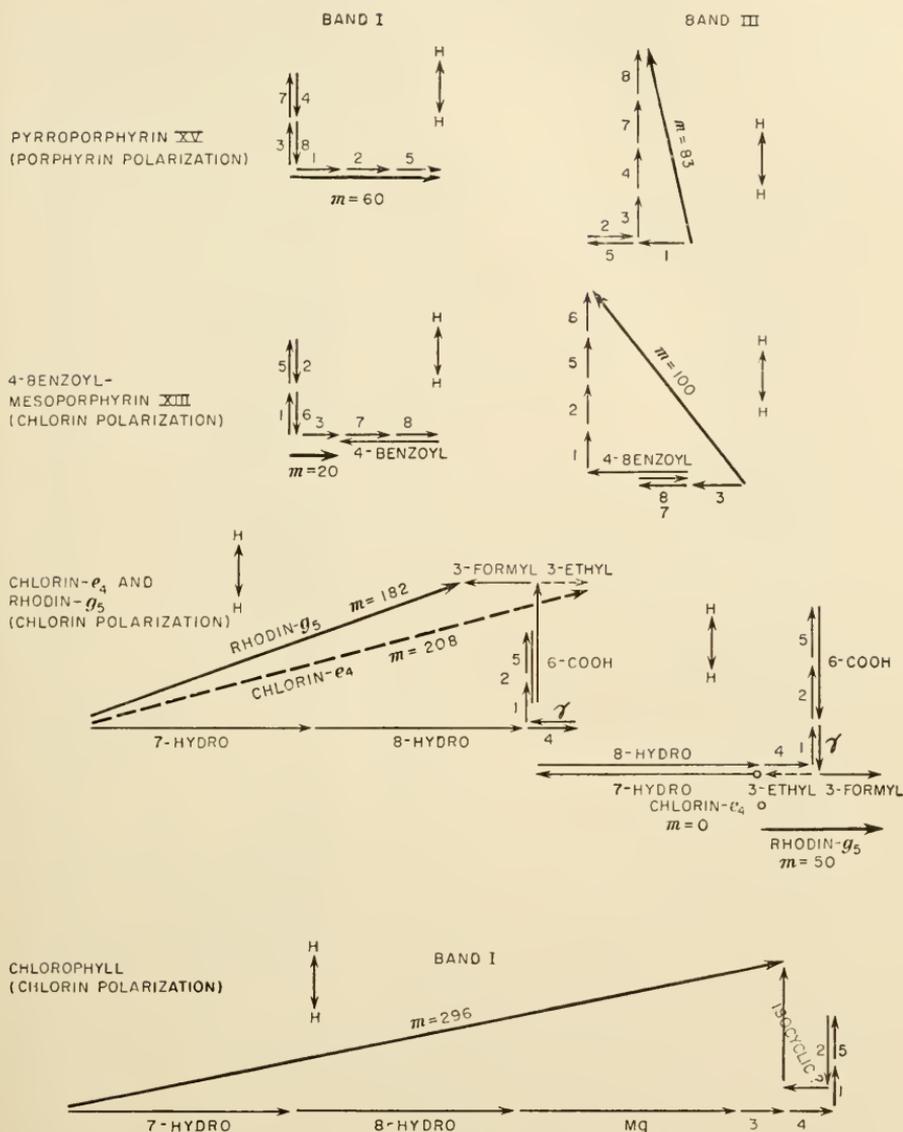


Fig. 2-21. Spectroscopic moment vector sums for typical polysubstituted porphyrins.

Then, adding the vectors in the directions given in Fig. 2-20 and squaring, we have, in general, for the change in intensity

$$I = (M_3 - M_4)^2 + (M_1 + M_2 + M_N - M_\alpha)^2 \quad (2-15)$$

for band I in porphyrin polarization and for band III in chlorin polarization; and

$$I = (M_1 - M_2)^2 + (M_3 + M_4 + M_N - M_\alpha)^2 \quad (2-16)$$

for band III in porphyrin polarization and for band I in chlorin polarization. For chlorins, these expressions simplify to

STRUCTURES OF COMPOUNDS IN FIG. 2-22

No. in fig.	Compounds	Stern <i>et al.</i> ref. No.	Substituents <sup>a</sup>						Polarization <sup>b</sup>
			12	34	56	78	$\alpha\beta\gamma\delta$	Metal	
1	Porphin	43, IV	—	—	—	—	—	—	P
2	1,5-dimethyl-2,6-diethyl-porphin	44, IV	22	—	22	—	—	—	C
3	Deuteroetioporphyrin II	47, IV	2-22	2-22	—	—	—	—	P
4	Deuteroporphyrin IX	46, IV	2-2	—	22	—	—	—	P
5	Pyrroporphyrin XV	8, I	22	22	2-22	—	—	—	P
6	$\gamma$ -Phylloporphyrin	15, II	22	22	2-22	—	—	—	P
7	Etioporphyrin I, II; eoproporphyrin I, II; mesoporphyrin IX; octaethylporphin	1-5, I; 14, II	22	22	22	—	—	—	P
8	$\alpha,\gamma$ -Dimethyl-(3)	48, IV	2-22	2-22	2-22	—	—	—	P
9	6-Vinyl-(5)	60, IV	22	22	23	22	—	—	C
10	6-Formyl-(5)	16, II	22	22	23	22	—	—	P
11	6-Acetyl-(5)	59, IV	22	22	23	22	—	—	P
12	6-Benzoyl-(5)	17, II	22	22	24	22	—	—	P
13	4-Benzoyl-mesoporphyrin XIII	50, IV	22	24	22	22	—	—	C
14	Rhodoporphyrin XV 6[-carboxyl-(5)]	11, I	22	22	25	22	—	—	P
15	6-Acrylic acid-(5)	61, IV	22	22	28	22	—	—	P
16	Dibenzoyldeuteroporphyrin XIII	49, IV	42	24	22	22	—	—	P
17	Chloroporphin- <i>e4</i> and - <i>e6</i>	21-2, II	22	22	25	22	—	—	P
18	$\gamma$ -Formyl-(14)	32, III	22	22	25	22	—	—	P
19	$\gamma$ -Carboxyl-(14)	64, IV	22	22	25	22	—	—	P
20	Rhodinoporphyrin- <i>gs</i>	63, IV	22	52	25	22	—	—	P
21	Pseudoverdoporphyrin	13, I	23	22	25	22	—	—	P
22	2-Cyclopropylcarboxylic acid-(14)	51, IV	22	22	25	22	—	—	P
23	4-Cyan-(4)	74, V	2-25	22	22	—	—	—	C
24	2-Acetyl-(14)	18, II	23	22	25	22	—	—	P
25	Oxochloroporphyrin- <i>e4</i>	62, IV	23	22	25	22	—	—	P
26	2,6-bisacetyl-(5)	58, IV	23	22	23	22	—	—	P
27	Protoporphylin	9, 10, I	23	23	22	22	—	—	P
28	Hemin	30, II	23	23	22	22	—	Fe	P
29	4-Formyl-(4)-oxime	69, V	2-23	22	22	—	—	—	P
30	6-Propionyl-(5)-oxime	67, V	22	22	23	22	—	—	C
31	2-Acetyl-(14)-oxime	68, V	23	22	25	22	—	—	P
32	Oxochloroporphyrin- <i>e4</i> -oxime	70, V	23	22	25	22	—	—	P
33	$\beta,\delta$ -diimino-eoproporphyrin II	57, IV	22	22	22	22	—	—	P

<sup>a</sup> Substituents designated as in Table 2-3, column "Code."

<sup>b</sup> P, porphyrin polarization; C, chlorin polarization; see Fig. 2-20.

$$I = (180 + m_3 + m_4 + M_N - M_\alpha)^2 \quad (2-15a)$$

for band I (neglecting the first term, which is relatively small) and

$$I = (m_3 - m_4)^2 + (M_1 + M_2 + M_N - M_\alpha)^2 \quad (2-16a)$$

for band III. For bacteriochlorins the formulas become

$$I = (360 + M_N - M_\alpha)^2 \quad (2-15b)$$

for band I and

$$I = (M_1 + M_2 + M_N - M_\alpha)^2 \quad (2-16b)$$

for band III; the numerical value in Eq. (2-15b) may need to be changed when more data are examined.

In Figs. 2-22 and 23 the resultant moments determined in this way are compared with the square root of the observed increase in intensity

above the unsubstituted intensity for bands I and III. Some "negative" values are shown, where it is supposed that the direction of the vectors is just reversed from the direction in adjacent compounds in the figure.

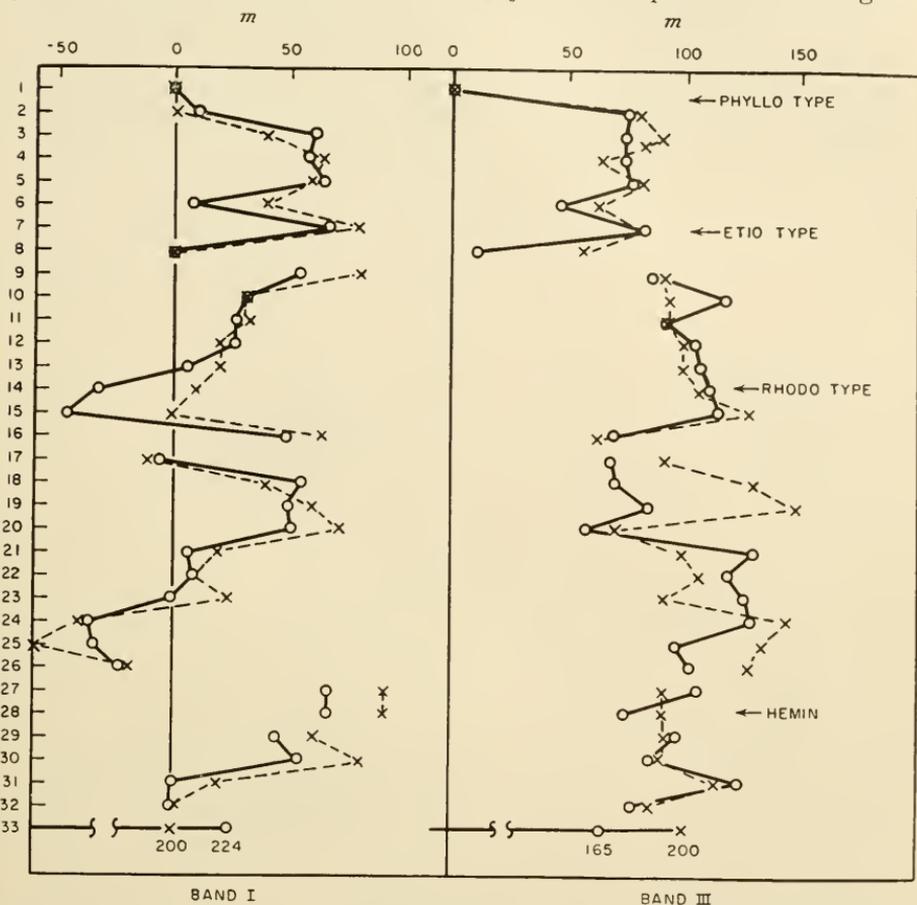


FIG. 2-22. Observed (o—o) and calculated (x---x) total spectroscopic moments for porphyrins. (For meaning of negative values, see text. For the diimino compound, 33, the points have been displaced to the left as indicated.)

It can be seen that many of the distinctive changes in the spectra are correctly reproduced, such as the differences between "phyllo," "etio," and "rhodo" types described by Stern and Wenderlein (1936, V). The "chlorin"- "rhodin" differences in the compounds examined in Fig. 2-23 are reproduced especially well. Chlorins have a positive  $m_3$  in Eq. (2-15a), and rhodins and *b*-compounds, negative, making their band I moments smaller by 50 units. The theory given here could also be applied to the porphyrins and chlorins studied by Dorough *et al.* (1951, 1952).

*Discrepancies.* The most conspicuous discrepancies are in those porphyrins with one strong acid substituent, such as rhodoporphyrin XV, its

derivatives, and its 6-acrylic acid analogue (compounds 14-26 in Fig. 2-22). Second-order effects with these strong moments might account for part of the discrepancy. But it is a firm prediction of the theory that the  $\gamma$ -alkyl derivative (chlorophorphyrin- $e_4$  and  $-e_6$ , compound 17), with a positive moment, and the  $\gamma$ -carboxyl derivative (compound 19), with a negative moment, should affect the rhodophorphyrin intensity oppositely. This is exactly contradicted by the fact that in band III both derivatives have intensities well below rhodophorphyrin XV itself.

STRUCTURES OF COMPOUNDS IN FIG. 2-23

No. in fig.	Compounds	Stern <i>et al.</i> ref. No.	Substituents <sup>a</sup>					Polarization <sup>b</sup>	
			12	34	56	78	$\alpha\beta\gamma\delta$		Metal
1	Chlorin- $e_4$ and $-e_6$	26-7, II	23	22	25	99	--2-	—	C
2	Dihydro-(1)	35-6, III	22	22	25	99	--2-	—	C
3	Rhodin- $g_5$ and $-g_7$	41-2, III	23	32	25	99	--2-	—	C
4	Rhodin- $g_3$	53, IV	23	32	2-	99	--2-	—	C
5	Rhodin- $g_7$ -oxime	75, V	23	32	25	99	--2-	—	C
6	Purpurin-7	82, V	23	22	25	99	--7-	—	C

With isocyclic rings (predictions tentative)

7	Pyropheophorbide <i>a</i> and pheophytin <i>a</i>	28, II; 52, IV	23	22	2*	99	--*--	—	C
8	Pheophorbide <i>a</i>	29, II	23	22	2#	99	--#--	—	C
9	Dihydro-(8)	39, III	22	22	2#	99	--#--	—	C
10	Methyl chlorophyllide <i>a</i>	76, V	23	22	2#	99	--#--	Mg	C
11	Pyropheophorbide <i>b</i>	40, III	23	32	2*	99	--*--	—	C
12	Pheophorbide <i>b</i>	37, III	23	32	2#	99	--#--	—	C
13	Dihydro-(12)	38, III	22	32	2#	99	--#--	—	C
14	2-Cyclopropyl-(12)	77, V	22	32	2#	99	--#--	—	C
15	2-Cyclopropyl-3-formyl-(12)-oxime	78, V	22	32	2#	99	--#--	—	C

<sup>a</sup> Substituents designated as in Table 2-3, "Code" column.

<sup>b</sup> P, porphyrin polarization; C, chlorin polarization; see Fig. 2-20.

\* Isocyclic ring —CH<sub>2</sub>—CO— between  $\gamma$  and 6 positions.

# Isocyclic ring —CH(COOCH<sub>3</sub>)—CO— between  $\gamma$  and 6 positions.

In view of the many other qualitative successes of the theory, as shown in Fig. 2-22, this direct qualitative contradiction suggests, at least to the author, that one of these compounds and possibly some of the other substituted rhodophorphyryns were either impure or misidentified. (This is known to have happened with a few of Stern and Wenderlein's compounds and is not surprising, since their measurements were made at a time when syntheses and structural determinations were still in a formative stage.)

*Isocyclic Rings and Metal Complexes.* The data are insufficient for determining uniquely the moments and directions for isocyclic rings connecting positions 6 and  $\gamma$ , such as occur in some of the most interesting compounds. We can still get a prediction of the band I intensity in pheophorbides and pheophytins, which have the bridge —CH<sub>2</sub>—CO— or —CH(COOCH<sub>3</sub>)—CO— between positions  $\gamma$  and 6. Stern and Wen-

derlein had pointed out that the absorption of these compounds is like that in the corresponding chlorin, which has a  $\gamma$ -methyl group and a 6-carboxyl ester substituent. This approximation is shown in Fig. 2-23 for band I and is satisfactory. It is worthless for band III, which is much more sensitive to the values and directions of the vector moments; consequently no predictions were made for the latter band.

To account for the intensity of band I of the chlorophylls or the methyl chlorophyllides, which are magnesium-phorbide complexes, we must assume that the magnesium substitution on the central nitrogens contributes a moment of value  $+90$ . Hemin, on the other hand, which is the iron-protoporphyrin complex, has almost the same spectrum as proto-

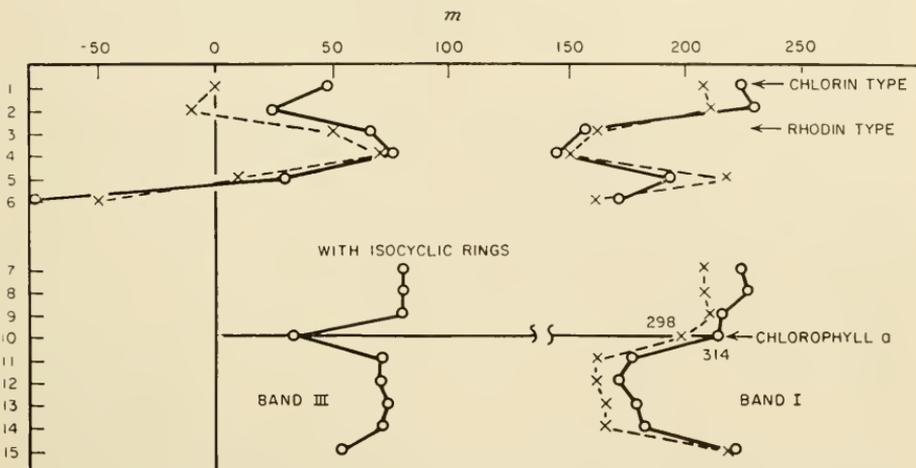


FIG. 2-23. Observed (o---o) and calculated (x---x) total spectroscopic moments for chlorins and rhodins. (For meaning of negative values, see text. For the chlorophyllide, 10, the points for band III have been displaced to the left as indicated.)

porphyrin itself, indicating that iron has a moment of about 0 and that it rather surprisingly does not change the  $D_{2h}$  symmetry.

The additional measurements of Stern *et al.* (Stern and Molvig, 1936; Stern and Pruckner, 1937a,b, 1939; Pruckner and Stern, 1936; Pruckner, 1940, 1941, 1942) still need to be examined to test this theory of the intensities.

The treatment given here derives the strong longest-wave-length transitions of the chlorins and bacteriochlorins by almost continuous stepwise perturbations from the weak porphyrin bands, so that all these compounds are encompassed by the same intensity formulas, Eqs. (2-15) and (2-16). It should be noted that this is in contradiction to the theory of Rabinowitch (1944). He proposed the introduction of new electronic transitions in the chlorins and bacteriochlorins to account for their strong red bands.

Wave-length shifts have not been discussed here. The shifts obviously go hand in hand with the intensity changes, but the theory of Förster

(1947) for the forbidden transition in benzene requires additional parameters to describe the wave-length shifts. His theory of this behavior can undoubtedly be adapted also to the porphyrin wave-length changes.

#### LIGHT EMISSION

Many reports of fluorescences and phosphorescences of porphyrins in the literature have been later refuted. It is hard to prove that positive results are not due to luminescent impurities, or that negative results are not due to quenching impurities. In fact it is known that  $10^{-5} M$  concentrations of certain polar impurities can produce marked changes in the spectra and fluorescence of chlorophyll solutions (Livingston *et al.*, 1949). But our concern in this chapter is with the positive results—with the location and properties of the energy levels so far as they can be determined.

Calvin and Dorough (1947) found a weak phosphorescence of chlorophyll b at 8600 Å, about  $4000 \text{ cm}^{-1}$  to the red of its  $^1Q_x$  band and  $6800 \text{ cm}^{-1}$  from  $^1Q_y$ , with a lifetime of  $3 \times 10^{-2}$  sec. A more definite result was obtained by the same authors (1948) on zinc tetraphenylchlorin, which had a phosphorescence at 8000 Å,  $3500 \text{ cm}^{-1}$  to the red of its  $^1Q_x$  band and  $5700 \text{ cm}^{-1}$  from  $^1Q_y$ , with a lifetime of about  $10^{-2}$  sec. The triplet upper state of these phosphorescences could be either  $^3Q_x$  or  $^3Q_y$ . The lifetime given is so short that it would surely be related to a strong singlet (Fig. 2-8), were it not for the fact that the heavy zinc atom will shorten the phosphorescence lifetime, as described earlier. In fact, the difference in the weight of the metal atoms may account for the differences in the intensities and lifetimes of the phosphorescences in these two compounds.

In the  $C_{18}$  and  $C_{22}$  condensed-ring hydrocarbons, the lowest triplet is always at an almost constant distance of about  $9000 \text{ cm}^{-1}$  below a "corresponding" singlet (Klevens and Platt, 1949), but whether this correspondence means that both states belong to the same orbital wave function is still being debated. In porphyrins the gap from the triplet to  $^1Q_y$  is more like this figure than the distance to  $^1Q_x$ , so that we may say that  $^1Q_y$  is probably the "corresponding" singlet. Provisionally we may label the triplet as  $^3Q_y$ . However, the separation from  $^1Q_x$  is still within the range of other triplet-singlet separations expected in condensed-ring systems. A determination of the phosphorescent-fluorescent separation in a porphin, a dihydroporphin, and a tetrahydroporphin would settle this question of the "correspondence." If the separation is constant, a correspondence of the triplet with  $^1Q_x$  is indicated; if it gets smaller (by  $2000$ – $4000 \text{ cm}^{-1}$ ) in the hydrocarbons, then the correspondence is with  $^3Q_y$ .

The changes in fluorescence found by Livingston and coworkers (1949) are more peculiar. They established that strictly dry chlorophyll a or b in a dry solvent probably has *no* fluorescence, but that minute quantities

of "activators" bring the fluorescence up to full strength in both compounds. These activators are polar substances that can form hydrogen bonds, e.g., water, alcohols, acids, or amines. They showed that the activators form a 1-to-1 complex with the chlorophyll and that the complex is the fluorescing agent.

The discussion in this chapter suggests a hypothesis that might account for this fluorescence behavior. It is that there is another electronic singlet state in dry chlorophyll a or b, below but close to the  ${}^1Q_x$  state, possibly a  ${}^1U_x$  or  ${}^1W_x$  state of the carbonyl group in the isocyclic ring of chlorophyll (phorbide type). Figure 2-12 shows that in a molecule the size of chlorophyll, with the  $\pi$ -transitions moved down to  $16,000\text{ cm}^{-1}$ , they might just be catching up with the carbonyl  $n$ -transitions. A low  ${}^1U$  or a low  ${}^1W$  state, with transitions to ground between 40 and 1000 times weaker than for  ${}^1Q_x$ , would be relatively nonfluorescent. (This would be a favorable situation for finding phosphorescence.) It would not be detected in absorption on the side of the strong  ${}^1Q_x$  band until it was several hundred wave numbers lower (see the left-hand side of Fig. 2-19); and it would move to the blue in the presence of polar molecules or protons, as described earlier in this chapter, perhaps passing above the  ${}^1Q_x$  and permitting the fluorescence to take place. Precisely such crossings between low  $n$ -transitions and  $\pi$ -transitions in polar solvents have now been identified for simpler molecules (McConnell, 1952).

The crossings, if they exist, could be of significance in biological porphyrins if they served to control the fluorescent and phosphorescent lifetimes and relative populations during irradiation.

The possibility that the phosphorescent triplet is  ${}^3U$  or  ${}^3W$  seems to be ruled out by the short reported lifetimes and by the small  $n$ - $g$  singlet-triplet separation calculated by Reid (1953).

If this explanation is correct, the peculiar fluorescence behavior is not directly related to the considerable changes in the absorption spectra which Livingston and coworkers (1949) also found between the dry and wet chlorophyll. (The shift in the  $n$ -transition would be almost undetectable.) Instead, these spectral changes are simply evidence of the change in the spectroscopic moment of the ring substituents when the activator complex is formed.

For porphyrins capable of forming such complexes, probably most of the spectra now in the literature should be ascribed to the complexed molecule, and spectroscopic moments determined from them should be attributed to the complex.

#### ELECTRON DENSITY AND EXCITATION

The  $\pi$ -electron densities in the ground and excited states of porphyrin and tetrahydroporphyrin are given in Table 2-4, as computed from the LCAO wave functions of Longuet-Higgins and coworkers (1950). These

TABLE 2-4.  $\pi$ -ELECTRON DENSITIES IN PORPHIN AND TETRAHYDROPORPHIN<sup>a</sup>  
 (ONE-ELECTRON APPROXIMATION)

Position		States					
Porphyrin polarization	Chlorin polarization	A	$\frac{W_x}{(U_x)}$	$Q_x$	$Q_y$	$B_y$	$B_x$
Porphin							
		Ground state	Excitation				
			$n-e_{yx}$	$a_{2u}-e_{yx}$	$a_{2u}-e_{yy}$	$a_{1u}-e_{yx}$	$a_{1u}-e_{yy}$
Rings on H-H axis	Rings on H-H axis						
3,4,7,8	1,2,5,6	1.085	<u>1.154</u>	<u>1.154</u>	1.087	1.120	1.053
N <sub>II</sub> , N <sub>IV</sub>	N <sub>I</sub> , N <sub>III</sub>	1.223	1.223	<u>1.098</u>	1.189	1.223	<u>1.314</u>
B <sub>II</sub> , B <sub>II'</sub> , } B <sub>IV</sub> , B <sub>IV'</sub> }	B <sub>I</sub> , B <sub>I'</sub> , } B <sub>III'</sub> , B <sub>III'</sub> }	1.071	1.100	1.100	1.074	<u>1.009</u>	<u>0.983</u>
$\alpha, \beta, \gamma, \delta$	$\alpha, \beta, \gamma, \delta$	0.966	<u>1.070</u>	0.945	0.945	<u>1.170</u>	<u>1.170</u>
Rings off H-H axis	Rings off H-H axis						
B <sub>I</sub> , B <sub>I'</sub> , } B <sub>III</sub> , B <sub>III'</sub> }	B <sub>II</sub> , B <sub>II'</sub> , } B <sub>IV</sub> , B <sub>IV'</sub> }	1.071	1.074	1.074	1.100	<u>0.983</u>	<u>1.009</u>
N <sub>I</sub> , N <sub>III</sub>	N <sub>II</sub> , N <sub>IV</sub>	1.223	<u>1.314</u>	1.189	<u>1.098</u>	<u>1.314</u>	1.223
1,2,5,6	3,4,7,8	1.085	<u>1.087</u>	1.087	<u>1.154</u>	1.053	1.120
Tetrahydroporphin							
		Ground state	Excitation				
			$n-b_{3g}$	$b_{1u}-b_{3g}$	$a_u-b_{3g}$	$b_{1u}-b_{2g}$	$a_u-b_{2g}$
	Rings on H-H axis						
	1,2,5,6	1.114	<u>1.180</u>	<u>1.180</u>	1.133	1.118	1.071
	N <sub>I</sub> , N <sub>III</sub>	1.276	1.276	<u>1.151</u>	1.276	1.241	<u>1.366</u>
	B <sub>I</sub> , B <sub>I'</sub> , } B <sub>III</sub> , B <sub>III'</sub> }	1.093	1.121	1.121	<u>1.037</u>	1.107	<u>1.023</u>
	$\alpha, \beta, \gamma, \delta$	1.062	<u>1.160</u>	1.035	<u>1.147</u>	<u>1.010</u>	<u>1.122</u>
	Rings off H-H axis:						
	B <sub>II</sub> , B <sub>II'</sub> , } B <sub>IV</sub> , B <sub>IV'</sub> }	1.056	1.059	1.059	<u>0.951</u>	<u>1.169</u>	1.061
	N <sub>II</sub> , N <sub>IV</sub>	1.075	<u>1.186</u>	1.061	<u>1.186</u>	<u>0.950</u>	1.075
	3,4,7,8	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)	(1.000)

<sup>a</sup> The following symbols are used in the table:

N<sub>I</sub>, the nitrogen on ring I; B<sub>I</sub>, either branch carbon of ring I; etc.

A bar over the density values indicates considerable loss of electrons in the excited state; a bar under the values, considerable gain.

Figures in parentheses in the bottom line show the effective  $\pi$ -electron density for hyperconjugation at the saturated atoms.

values are for the "one-electron approximation," neglecting the mixing that has been postulated here between the one-electron  $Q_x$  and  $B_x$  states and between the one-electron  $Q_y$  and  $B_y$  states. (The symbols  $Q$  and  $B$  do not properly apply to one-electron wave functions but to the mixed functions; we use them here only for brevity.)

It is evident from the table that excitation from the ground state of porphin to  $Q_x$  involves electronic charge transfer principally within the two rings that are on the H-H axis, from the nitrogen in those rings to the external carbons. (This should excite strongly the corresponding pyrrole vibration.) When  $Q_x$  is mixed with  $B_x$ , charge will move from the branch carbons to restore some of the nitrogen loss and will also build up on the  $\alpha$  positions. The total result in the mixed  $Q_x$  state is activation of the  $\alpha$  positions and of the external carbons on the H-H rings.

Excitation from the ground state to  $Q_y$  correspondingly produces activation of the external carbons on the other two rings, those perpendicular to the H-H axis, and when  $Q_y$  is mixed with the  $B_y$  state, excitation of the  $\alpha$  positions as well.

In the  $U_x$  or  $W_x$  state, a nonbonding electron would go principally to the  $\alpha$  positions, to the end carbon atoms on the H-H axis, and to the nitrogen atoms off it. In Table 2-4 tetrahydroporphin shows much the same behavior, except that the  $Q_y$  and  $B_y$  properties are interchanged. The end carbon atoms on the H-H axis are activated in  $Q_x$  excitation, and the  $\alpha$  positions also, when  $B_x$  is mixed in, with the branch carbon atoms on the end rings losing charge. In  $Q_y$  excitation, charge moves from the branch carbon atoms to the  $\alpha$  positions and the side nitrogens, but mixing of  $Q_y$  with  $B_y$ , since there are now no side rings to acquire charge, tends to restore approximately the ground-state electron density.

In the  $U_x$  or  $W_x$  state a nonbonding electron would go principally to the  $\alpha$  positions, the end carbon atoms, and the side nitrogen atoms. In porphin, then, the singlet or triplet  $U_x$ ,  $W_x$ , or  $Q_x$  (mixed) states should favor proton addition at the carbon atoms in the rings on the H-H axis; the  $U_y$ ,  $W_y$ , or  $Q_y$  states, in the rings off the axis. In tetrahydroporphin the latter states would not be especially favorable to electrophilic additions except possibly at the  $\alpha$  positions, but additions at these points would separate the conjugated system into two less stable parts and so might easily be lost again when the ground state was reached. The singlet and triplet  $U_x$ ,  $W_x$ , and  $Q_x$  states have a high electron density at the end carbon atoms and would attract two protons there, but again the protons cannot stay, for this would produce a highly unstable 17-membered ring (with amino group attached) or, from another point of view, a highly unstable 16-membered ring (with vinyl group attached).

*Photooxidation.* In tetrahydroporphin, on the other hand, Table 2-4 shows that the  $\pi$ -electron density on the side rings is abnormally low, particularly at the saturated carbon atoms and, in the  $Q_y$  singlet and

triplet states, also at their neighbors, the branch carbon atoms. (The mixing with the  $B_y$  one-electron wave function should increase the branch-carbon density again, but this mixing, which was strong in porphin, will be relatively small in the tetrahydro compound because of the loss of square symmetry.) This is, then, a relatively favorable situation for the loss of protons from the saturated carbons, especially since the resultant conjugated system become larger.

We may infer that dihydroporphins must have an intermediate behavior and so will be unusual in their simultaneous capacity for photoreduction—adding protons to one side ring in their phosphorescent state—and for photooxidation—giving up protons from the other side ring—at least wherever the available excitation energy of about 40 kcal/mole is sufficient for the reaction. These calculations might be somewhat related to the unique biological role of these compounds as photocatalysts. Probably the successful quantitative treatment by Longuet-Higgins (1950b,c) of proton-addition problems in amino aromatics from theoretical electron densities could be adapted to the present problem, and the additional directing effects of the ring substituents, which we have neglected here, could also be considered.

Calvin and Dorough (1948) showed convincingly that zinc tetraphenylchlorin in its phosphorescent state could give its excess hydrogen atoms to oxygen and to quinones and be converted to the porphin. A related process may be the reversible photochemical interaction between chlorophyll and quinone at low temperatures in rigid glassy solvents, which was found by Linschitz and Rennert (1952). Since this process should be most probable in the  $Q_y$  state, the result is not in disagreement with our provisional labeling of the phosphorescent state as  ${}^3Q_y$ . It would be interesting to find out whether porphin in its phosphorescent state would give the reverse reaction, as supposed here, and, for a substituted porphin, on which rings the added hydrogen atoms would go. It would also be interesting to know whether the chlorin in its phosphorescent state can take hydrogen atoms from a suitable donor before it has lost any.

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## CHAPTER 3

# Generation, Control, and Measurement of Visible and Near-visible Radiant Energy

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*Fundamental concepts: Nomenclature—Electromagnetic spectrum—Quantitative units of energy—Propagation of radiant energy—Absorption of radiant energy—Reflection—References. Sources of radiant energy: Thermal sources—Gaseous discharge lamps—Fluorescent lamps—Short-duration sources—References. Control of radiant energy: Optical properties of materials—Spectral control of radiant flux—Control of irradiance—References. Measurement of radiant energy: Detectors—Measuring instruments—Standards of radiant energy—Voltage regulators—References.*

In the investigation of the biological role of radiant energy, one must consider both the complexity of photochemical processes in biological systems and the proper selection and application of appropriate technics from the great diversity of modern physical methods of radiation production, control, and measurement.

Rapid technological development has markedly increased the experimental opportunities in the field of photochemical biology. Many diversified sources of radiant energy are available. Detectors such as the photomultiplier make it possible to measure extremely low levels of visible and ultraviolet energy, and photoconductive cells such as the lead sulfide cell extend the spectral range of photocell detectors well into the infrared. Great strides have been made in electrical measurements, probably the most striking of which are inverse feedback circuitry, servo-control, and the various modulation systems for converting low-level direct currents to alternating currents capable of being amplified readily by electronic means. Fast thermal detectors, such as thermocouples, bolometers, and pneumatic cells, have also made it possible to modulate the radiant-energy beam itself. Such systems of modulation permit the substitution of a-c vacuum-tube amplifiers and strip-chart recorders for galvanometers and photographic methods of recording.

The present chapter represents an effort to assemble the most pertinent information on modern developments, together with the most important of the fundamental principles and technics of application of visible and near-visible radiant energy to biological problems.

## 1. FUNDAMENTAL CONCEPTS

Radiant energy is that form of energy which is propagated through space as electromagnetic waves. On interaction with matter, such energy behaves not as a continuum, but as a series of indivisible discrete packets known as "quanta" or "photons." Our present discussion concerns the properties and experimental technics pertaining to that region of the spectrum in which the photons have sufficient energy to alter the outer electronic energy levels of atoms but not sufficient energy for complete ionization, thus encompassing only the near ultraviolet, the visible, and the very near infrared (Brackett, 1936; Daniels, 1948; Forsythe, 1937; Habell and Cox, 1948; Illuminating Engineering Society, 1952; Richtmyer and Kennard, 1947).

### NOMENCLATURE

Before entering into a full discussion of the fundamental concepts of radiant energy, it is desirable to present an outline of the nomenclature to be subsequently employed, particularly since the complexity of the terminology has led to much confusion and the inconsistent use of terms (Withrow, 1943). The Illuminating Engineering Society of America (1942, 1952); the Optical Society of America, Committee on Colorimetry (1944a,b,c, 1953); and the National Bureau of Standards, through Brode (1949), Gibson (1949), and Judd (1950), have been especially active in the United States in developing a standardized nomenclature.

Within the visible spectrum, radiant energy may be evaluated either as a physical entity by the use of physical detectors or as a psychophysical entity by visual means. Thus two systems of nomenclature have been evolved, one dealing with the evaluation of radiant energy as a purely physical entity (radiometric terms) and the other with psychophysical evaluation (photometric terms). This dual nomenclature has led to the frequent use of photometric quantities as substitutes for radiometric terms in technical writing. Such misuse undoubtedly results from the tendency of the investigator to extend terms applicable to his own visual experiences to the experimental system under consideration, but this practice frequently leads to ambiguity in scientific writing.

The physical and psychophysical radiation terms are compared and briefly defined in Table 3-1. At the bottom of the table are given the connotations of the various prefixes and suffixes used; these are consistent with those employed generally in scientific nomenclature. In Table 3-2 defining equations and symbols are given for the more commonly used quantitative radiometric and photometric terms.

Certain terms are misused more than others and merit special mention. Although the nomenclature committees make a distinction between light and luminous energy, for practical purposes these may be considered as synonymous with radiant energy evaluated over the luminosity curve of

the human eye. On this basis, "ultraviolet light," "infrared light," "black light," and similar terms are misnomers that have no place in precise scientific writing, although they may be useful in nontechnical discussions. In place of the term "light" for the physical entity, it is more consistent to use "radiant energy" or simply "energy." When

TABLE 3-1. COMPARISON OF PHYSICAL AND PSYCHOPHYSICAL  
RADIANT-ENERGY TERMS

Physical	Psychophysical	Defining statement
Radiant energy.....	Luminous energy, light	Physical or psychophysical entity
Radiant flux.....	Luminous flux, light	Time rate of flow of energy, power
Radiation.....	Lumination	Process of generation of energy
Radiator, source, lamp...	Luminator, source, lamp	Generating device, source of energy
Radiant emittance.....	Luminous emittance	Flux radiated per unit area of source
Radiant intensity.....	Luminous intensity, candlepower	Flux radiated per unit solid angle
Radiance.....	Luminance, brightness	Flux radiated per unit area and solid angle
Irradiation.....	Illumination	Process of interception of energy
Irradiance.....	Illuminance	Flux intercepted per unit area
Radiometry.....	Photometry	Science of measurement

## Prefixes:

- radi-* pertaining to radiant energy
- photo-* pertaining to luminous energy but also applied to radiant energy, as in "photograph" and "photon"
- lumi-* pertaining only to luminous energy
- irradi-* concerning interception of radiant flux
- illumi-* concerning interception of luminous flux

## Suffixes:

- ion* process, as in "conduction" or "calibration"
- er, -or* device, as in "generator" or "absorber"
- ance* measurable property, as in "resistance" or "reflectance"
- ity* specific property as in "density" or "conductivity"

the concept of power is involved, "radiant flux" or "flux" is a suitable term. Radiation implies the process of generation of radiant energy and is not synonymous with radiant energy, although it is frequently so used, especially by those dealing with ionizing radiant energy. "Radiant emittance," "radiant intensity," and "radiance" are intensity terms applicable to sources in that they are related to the radiant power per unit area and/or solid angle. "Irradiance" is the intensity term applicable to the interception of radiant energy by objects and is power per unit area. Frequently "intensity" is used loosely as a substitute for

TABLE 3-2. RADIOMETRIC AND PHOTOMETRIC TERMS AND UNITS

Radiometry			Photometry		
Term	Symbol <sup>a</sup> and defining equation	Unit	Term	Symbol <sup>a</sup> and defining equation	Unit
Radiant energy.....	$U$	erg, joule, caloric	Luminous energy.....	$Q$	lumerg, talbot
Radiant density.....	$u = dU/dV$	erg cm <sup>-3</sup>	Luminous density.....	$q = dQ/dV$	lumerg cm <sup>-3</sup>
Radiant flux.....	$P = dU/dt$	erg sec <sup>-1</sup> , watt	Luminous flux.....	$F = dQ/dt$	lumen
Radiant emittance.....	$W = dP/dA$	watt cm <sup>-2</sup>	Luminous emittance.....	$L = dF/dA$	lumen cm <sup>-2</sup>
Radiant intensity.....	$J = dP/d\omega$	watt $\omega^{-1}$	Luminous intensity, candlepower.....	$I = dF/d\omega$	lumen $\omega^{-1}$ (cand- dlc)
Radiance.....	$N = \frac{dP}{d\omega(dA \cos \theta)}$	watt $\omega^{-1}$ cm <sup>-2</sup>	Luminance, brightness.....	$B = \frac{dF}{d\omega(dA \cos \theta)}$	candle $\omega^{-1}$ cm <sup>-2</sup> , lambert
Irradiance.....	$H = dP/dA$	erg sec <sup>-1</sup> cm <sup>-2</sup> , watt cm <sup>-2</sup>	Illuminance, illumination.....	$E = dF/dA$	lumen m <sup>-2</sup> lux lumen ft <sup>-2</sup> , foot- candle

<sup>a</sup> Symbols:  $V$  = volume, $A$  = area, $t$  = time, $\theta$  = angle (radians), $\omega$  = solid angle (steradians).

“irradiance.” The psychophysical terms “illumination” and “brightness” are often used erroneously in connection with nonvisual applications. It is seldom that the biologist has occasion to use such terms except when dealing specifically with the eye, with vision, or with visual instruments such as the polarimeter or the visual colorimeter.

Several inconsistent photochemical expressions also are widely used. “Light reaction” should be limited to the psychophysics of vision; “photoreaction,” “photochemical reaction,” and “photoprocess” are preferable physical terms. The term “dark reaction” as used in photochemistry usually refers to a nonphotochemical reaction that may or may not proceed in a radiation field. It may be thermochemical, thus requiring high-energy collisions that are a function of temperature, or it may be a physical process, as in diffusion. It seldom is, as the expression implies, a reaction that takes place only in the absence of radiant energy. “Nonphotochemical” reaction is a more meaningful general term, and such terms as “thermochemical” reaction and “diffusion” reaction are more specific.

#### ELECTROMAGNETIC SPECTRUM

The electromagnetic spectrum is an orderly arrangement of radiant energy according to wave length and frequency and extends from the very-long-wave low-energy photons of the radio region, as produced by oscillatory electrical circuits, to the extremely high-energy particles of the short-wave cosmic rays; this is presented graphically on a logarithmic scale in Fig. 3-1. There is a continuous transition in physical properties in passing through the spectrum, even though each spectral region is frequently treated as if it were a sharply defined entity.

The various spectral regions have been somewhat arbitrarily delimited by certain of their most evident properties. The visible spectrum extends from about 380  $m\mu$  in the violet to 770  $m\mu$  in the red, as determined by the limits of the spectral sensitivity of the average light-adapted human eye (see Fig. 3-2). The near ultraviolet is usually taken as the region from the short-wave-length limit of the visible to the furthest limit of transmission of optical glass at about 320  $m\mu$ ; the spectrum of the sun extends slightly further to about 290  $m\mu$ . The far ultraviolet continues to 180  $m\mu$ , where quartz and air begin to absorb strongly. Beyond this is the vacuum or extreme ultraviolet, which can be studied only in evacuated systems.

On the long-wave-length side of the visible is the infrared, which goes from about 770  $m\mu$  to thousands of microns. The region of principal biological interest, however, is limited to the very near infrared between 770 and 1500  $m\mu$ . Since water begins to absorb very strongly beyond 1500  $m\mu$ , most biological materials are quite opaque to energy of longer wave lengths. Here the energy of the quantum is too low to affect the electronic energy levels of the atoms, and only the rotational, vibrational,

and translational energy levels are changed; therefore few if any photochemical reactions occur here, and the radiant energy is degraded to heat.

The infrared has been commonly referred to as the "heat-radiation" region of the spectrum because most of the energy radiated by all high-intensity sources is in the infrared. However, the energy of all spectral regions is ultimately degenerated to heat when absorbed by matter and is

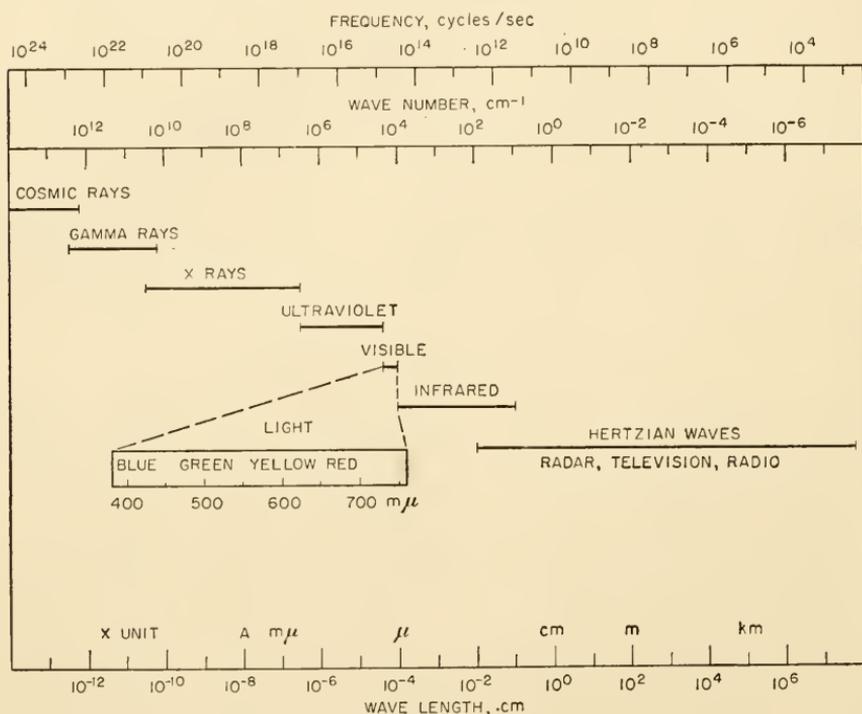


FIG. 3-1. The electromagnetic spectrum.

the basis for the quantitative measurement of radiant energy by thermal detectors. The infrared is intrinsically no more a region of "heat radiation" than any other spectral region.

#### SPECTRAL UNITS

Radiant energy may be characterized quantitatively by either wave length or frequency. For any periodic wave motion the wave length is defined as the linear distance between two similar points on adjacent waves, such as the distance from crest to crest or trough to trough. The frequency is the number of waves passing a given point in an interval of time, which is usually the second.

In a vacuum, electromagnetic waves have a velocity (the velocity of light) which is never exceeded in value and is a universal constant. In accordance with the laws of wave motion, the velocity is equal to the

product of wave length and frequency:

$$c = \nu\lambda, \text{ or } \lambda = c/\nu, \quad (3-1)$$

where  $c$  = velocity of electromagnetic waves in a vacuum,  $2.998 \times 10^{10}$  cm/sec,

$\nu$  = frequency, waves per second, and

$\lambda$  = wave length, centimeters per wave.

The three most commonly used units of wave length in the visible and near-visible regions are the micron, the millimicron, and the angstrom. Their relations to the millimeter and the meter are:

$$\begin{aligned} \text{micron, } \mu &= 10^{-3} \text{ mm} = 10^{-6} \text{ m} \\ \text{millimicron, m}\mu &= 10^{-6} \text{ mm} = 10^{-9} \text{ m} \\ \text{angstrom, } \text{\AA} &= 10^{-7} \text{ mm} = 10^{-10} \text{ m} \\ 1 \mu &= 1000 \text{ m}\mu = 10,000 \text{\AA} \\ 1 \text{ m}\mu &= 10 \text{\AA} \end{aligned}$$

TABLE 3-3. COMPARISON OF SPECTRAL UNITS OF RADIANT ENERGY

Wave length, $m\mu$	Wave number, $\text{cm}^{-1}$	Frequency, $\text{sec}^{-1} (\times 10^{12})$
200	50,000	1500
250	40,000	1200
300	33,000	1000
350	28,600	857
400	25,000	750
450	22,200	667
500	20,000	600
550	18,200	545
600	16,700	500
650	15,400	462
700	14,300	429
750	13,300	400
800	12,500	375
850	11,800	353
900	11,100	333
950	10,500	316
1000	10,000	300
1050	9,520	286
1100	9,090	273
1150	8,700	261

The micron is employed usually in the infrared and occasionally in the visible; the millimicron and angstrom are used principally in the visible and ultraviolet. The angstrom is employed more commonly by physi-

cists, whereas the millimicron is preferred by biologists and chemists where three significant figures are adequate.

Interference and diffraction methods of characterizing radiant energy yield data that are proportional to wave length, and most absorption spectra in the visible and ultraviolet are plotted as a function of some wave-length unit. However, as related to molecular structure, a frequency unit is a more logical basis of specification, because the energy of the quantum is proportional to frequency, and visible and infrared absorption spectra display maxima that occur at simple multiples of fundamental frequency units. Frequency may be expressed directly as waves per second  $\nu$  or the fresnel  $f$ , which is  $10^{12}$  waves per second. However, the most commonly used unit, proportional to frequency, is the wave number  $\nu'$ , which is the number of waves per centimeter path in a vacuum and is expressed in reciprocal centimeters,  $\text{cm}^{-1}$ , such that

$$\nu' = 1/\lambda = \nu/c. \quad (3-2)$$

$\lambda\nu' = 10^7$  when  $\lambda$  is in millimicrons and  $\nu$  is in reciprocal centimeters. Comparison of the various spectral units at 50-m $\mu$  intervals of wave length is presented in Table 3-3.

#### QUANTUM ENERGY

In addition to wave length and frequency, radiant energy may be characterized by the energy of its smallest element, the quantum, or photon. According to the quantum theory, the energy of the photon  $\epsilon$  in ergs is equal to the product of Planck's constant  $h$ , which has a value of  $6.624 \times 10^{-34}$  joule-sec, and the frequency  $\nu$  in reciprocal seconds, as follows:

$$\epsilon = h\nu = h\nu'c = hc/\lambda. \quad (3-3)$$

The quantum, or photon, energy is proportional to frequency and inversely proportional to wave length. Since the individual molecule is too small a unit for experimental treatment, the einstein, or mole of quanta, is used:  $E = Nh\nu$ , where  $N$  is Avogadro's number,  $6.02 \times 10^{23}$  molecules per gram-molecule. Thus for 1 gram-molecule of substance photochemically reacted in the primary process, 1 einstein of photons is required.

Whereas the chemist usually expresses energy in gram-calories per gram-molecule, the physicist and the theoretical chemist are inclined to use the electron volt. The magnitude of the electron volt is derived from the kinetic energy the electron receives in dropping through an electrostatic field of 1 v; thus  $\frac{1}{2}mv^2 = eV$ .

The energy that an electron, having a charge of  $1.602 \times 10^{-19}$  coulomb, acquires when it interacts with a photon and absorbs the whole quantum of energy can be equated to the equivalent potential through which the

electron would have to fall to obtain the same energy; therefore

$$eV = hc/\lambda,$$

and

$$V = hc/e\lambda. \quad (3-4)$$

$V = 1240/\lambda$  ev when  $\lambda$  is in millimicrons. Table 3-4 gives the values of

TABLE 3-4. QUANTUM ENERGY OF VARIOUS WAVE LENGTHS OF RADIANT ENERGY

Wave length, $m\mu$	ev	Ergs/quantum ( $\times 10^{-12}$ )	Joules/einstein (or mole of quanta) ( $\times 10^5$ )	kg-cal/einstein (or mole of quanta)
200	6.25	9.93	5.98	142.9
250	5.00	7.94	4.78	114.2
300	4.17	6.62	3.99	95.1
350	3.57	5.67	3.42	81.5
400	3.12	4.96	2.99	71.5
450	2.78	4.41	2.66	63.6
500	2.50	3.97	2.39	57.1
550	2.27	3.61	2.17	51.9
600	2.08	3.31	1.99	47.6
650	1.92	3.06	1.84	44.0
700	1.79	2.84	1.71	40.9
750	1.67	2.65	1.60	38.2
800	1.56	2.48	1.50	35.6
850	1.47	2.34	1.41	33.7
900	1.39	2.20	1.33	31.5
950	1.32	2.09	1.26	30.1
1000	1.25	1.99	1.20	28.7
1050	1.19	1.89	1.14	27.2
1100	1.14	1.80	1.09	26.0
1150	1.09	1.73	1.04	24.9

the quantum energies at 50- $m\mu$  wave-length intervals in various commonly used units. Table 3-5 presents the number of quanta or einsteins in each unit of radiant energy (Daniels, 1948).

#### QUANTUM YIELD

The need for expressing absorbed energies in quantum units stems from the application of Einstein's law of photochemical equivalence, which states that in the primary photochemical reaction 1 quantum is absorbed for each atom or molecule reacted. Thus the number of molecules photochemically activated must equal the number of quanta absorbed, and therefore the number of moles reacted must be equal to the number of einsteins (moles of quanta) absorbed. The experimental data for most photochemical reactions seldom show the simple one-to-one

correspondence between photons absorbed and molecules reacted as required by Einstein's law. This is not the result of failure of the law but is due to complicating side reactions peculiar to each process. The

TABLE 3-5. NUMBER OF QUANTA OR EINSTEINS PER UNIT OF RADIANT ENERGY FOR VARIOUS WAVE LENGTHS

Wave length, m $\mu$	Quanta/erg ( $\times 10^9$ )	Quanta/g-cal ( $\times 10^{17}$ )	Einsteins/joule ( $\times 10^{-8}$ )	Einsteins/g-cal ( $\times 10^{-6}$ )
200	101	42	168	7.0
250	126	53	209	8.7
300	151	63	251	10.5
350	176	74	292	12.2
400	202	84	334	14.0
450	226	94	375	15.7
500	252	105	419	17.5
550	277	116	460	19.2
600	302	126	502	21.0
650	327	137	543	22.7
700	352	147	585	24.4
750	377	158	626	26.2
800	403	168	671	28.0
850	427	178	709	29.6
900	454	190	758	31.7
950	478	200	794	33.2
1000	502	210	834	34.9
1050	529	221	877	36.7
1100	552	231	917	38.5
1150	578	242	962	40.1

measure of the effectiveness of the radiant energy is given by the quantum efficiency, or quantum yield,  $\phi$  [denoted as  $\gamma$  by Rabinowitch (1945)]:

$$\phi = \frac{\text{number of molecules reacting}}{\text{number of quanta absorbed}}$$

In photosynthesis the reciprocal of the quantum efficiency  $1/\phi$  is termed the "quantum requirement" and is the number of quanta per reacting molecule.

#### QUANTITATIVE UNITS OF ENERGY

The quantitative units of radiant energy fall into three categories: (1) energy, which is related to the total quantity of the radiant energy; (2) power, the time rate of flow of energy, or energy per unit of time; and (3) intensity, the power per unit area, volume, and/or solid angle.

There are two principal systems of metric units: the centimeter-gram-

second (cgs) and the meter-kilogram-second (mks). The mks system is replacing the cgs system of units in physics and engineering because the mks units are more suitable for practical use. An excellent discussion of the various systems is given by Crittenden (1944, 1950).

TABLE 3-6. CONVERSION FACTORS OF RADIANT ENERGY, POWER, AND INTENSITY UNITS

Energy	erg	joule	g-cal	whr	kg-cal
erg, d-cm.....	1	$10^{-7}$	$0.239 \times 10^{-7}$	$0.278 \times 10^{-10}$	$0.239 \times 10^{-10}$
joule, w-sec.....	$10^7$	1	0.239	$0.278 \times 10^{-3}$	$0.239 \times 10^{-3}$
g-cal.....	$4.19 \times 10^7$	4.19	1	$1.163 \times 10^{-3}$	$10^{-3}$
whr.....	$3.60 \times 10^{10}$	3600	860	1	0.860
kg-cal.....	$4.19 \times 10^{10}$	4190	1000	1.16	1
Power	erg sec <sup>-1</sup>	$\mu$ w	cal min <sup>-1</sup>	w	cal sec <sup>-1</sup>
erg sec <sup>-1</sup> .....	1	0.1	$1.43 \times 10^{-6}$	$10^{-7}$	$0.239 \times 10^{-7}$
$\mu$ w.....	10	1	$1.43 \times 10^{-5}$	$10^{-6}$	$0.239 \times 10^{-6}$
cal min <sup>-1</sup> .....	$6.98 \times 10^5$	$6.98 \times 10^4$	1	0.0698	0.0166
w.....	$10^7$	$10^6$	14.3	1	0.239
cal sec <sup>-1</sup> .....	$4.19 \times 10^7$	$4.19 \times 10^6$	60	4.19	1
Intensity	erg sec <sup>-1</sup> cm <sup>-2</sup>	$\mu$ w cm <sup>-2</sup>	$\mu$ w mm <sup>-2</sup>	w m <sup>-2</sup>	cal min <sup>-1</sup> cm <sup>-2</sup>
erg sec <sup>-1</sup> cm <sup>-2</sup> .....	1	0.1	0.001	0.001	$1.43 \times 10^{-6}$
$\mu$ w cm <sup>-2</sup> .....	10	1	0.01	0.01	$1.43 \times 10^{-5}$
$\mu$ w mm <sup>-2</sup> .....	1000	100	1	1	$1.43 \times 10^{-3}$
w m <sup>-2</sup> .....	1000	100	1	1	$1.43 \times 10^{-3}$
cal min <sup>-1</sup> cm <sup>-2</sup> .....	$6.98 \times 10^5$	$6.98 \times 10^4$	698	698	1
Brightness	foot-lambert	lambert	c cm <sup>-2</sup>	c mm <sup>-2</sup>	
foot-lambert.....	1	$1.08 \times 10^{-3}$	$3.39 \times 10^{-3}$	$3.39 \times 10^{-5}$	
lambert.....	929	1	0.318	$0.318 \times 10^{-3}$	
c cm <sup>-2</sup> , stilb.....	2920	3.14	1	0.01	
c mm <sup>-2</sup> .....	$2.92 \times 10^5$	314	100	1	
Illuminance	lux	ft-c	lumen cm <sup>-2</sup>		
lux, m-c.....	1	0.093	$10^{-4}$		
ft-c.....	10.8	1	$1.08 \times 10^{-3}$		
lumen cm <sup>-2</sup> , phot.....	$10^4$	929	1		

The basic unit of energy in the cgs system is the erg, defined as the work done when a force of 1 dyne is applied through a distance of 1 cm. The dyne is the force required to give a 1-g mass an acceleration of 1 cm/sec<sup>2</sup>. The energy unit of the mks system is the joule, which is

the work done when a force of 1 newton acts through a distance of 1 m. The newton is the force required to give a 1-kg mass an acceleration of 1 m/sec<sup>2</sup>. The joule is equivalent to 10<sup>7</sup> ergs. The gram-calorie, or small calorie, is a unit of heat which is the energy (heat) required to raise 1 g of water 1°C in the interval from 15° to 16°C. The watt is the mks unit of power and is equivalent to 1 joule/sec or 10<sup>7</sup> ergs/sec. The microwatt is equal to 10 ergs/sec.

#### RADIOMETRIC UNITS

The conversion factors for the various units of energy, power, and intensity presented in Table 3-6 are arranged in the order of increasing size for convenience in comparison. Since the mks system has many

TABLE 3-7. MULTIPLYING PREFIXES FOR QUANTITATIVE PHYSICAL UNITS

Prefix	Symbol	Factor	Example
mega-.....	M	10 <sup>6</sup>	megacycle
kilo-.....	k	10 <sup>3</sup>	kilogram
hecto-.....	h	10 <sup>2</sup>	hectowatt
deka-.....	dk	10	dekaliter
deci-.....	d	10 <sup>-1</sup>	deciliter
centi-.....	c	10 <sup>-2</sup>	centimeter
milli-.....	m	10 <sup>-3</sup>	millimeter
micro-.....	μ	10 <sup>-6</sup>	microgram
nano-.....	n	10 <sup>-9</sup>	nanogram
pico-.....	p	10 <sup>-12</sup>	picogram

practical advantages for general work and since the Bureau of Standards calibrates its radiation standard sources in microwatts per square centimeter of irradiance at a specified source distance, the mks system is considered preferable for radiant-energy measurement. Thus the microjoule may be used in place of the erg (1 μj = 10 ergs), and the microwatt per square centimeter in place of the erg per second per square centimeter. It will be noted that the watt per square meter is equivalent to the microwatt per square millimeter and to 100 μw cm<sup>-2</sup>. The U.S. Weather Bureau, probably for historical reasons, still uses the calorie per minute per square centimeter for the specification of solar irradiance, but the watt per square meter is now often preferred. The multiplying factors for the various prefixes used with physical units are given in Table 3-7. The prefixes "nano-" and "pico-" have been recommended by the symbols committee of the Royal Society of London (Pirie, 1951).

#### PHOTOMETRIC UNITS

Units of illuminance (Table 3-6) are based on the international candle as the unit of luminous intensity (Illuminating Engineering Society, 1952;

Optical Society of America, 1953). The primary standard of luminance is a complete, or black-body, radiator maintained at  $2044^{\circ}\text{K}$  by freezing platinum and has an illuminance of  $60 \text{ c cm}^{-2}$ .

The lumen is a unit of luminous flux such that a point source with a luminous intensity of 1 candle produces a total luminous flux of  $4\pi$  lumens, or 1 lumen  $\omega^{-1}$ . The steradian is that solid angle which encloses a surface on a sphere equivalent to the square of the radius. Thus a sphere contains  $4\pi$  steradians, since the area of the surface is  $4\pi r^2$ . The steradian is a dimensionless unit.

The mks unit of illuminance is the lux and is  $1 \text{ lumen m}^{-2}$ . The foot-candle, which is nearly 11 times as large as the lux, is  $1 \text{ lumen ft}^{-2}$ . The lux is the preferable unit for scientific use, but since many commercial illumination meters are calibrated in the foot-candle, this unit is widely used in the United States.

The units of brightness or luminous intensity have the same dimensions as those for illuminance or illumination but have different names. The units may be applied to real sources, such as the filament of an incandescent lamp, or to virtual sources, such as an illuminated surface. Since  $4\pi$  lumens is emitted by a source of 1 candle, it can be shown by integration that a source of 1 candle per unit area emits  $\pi$  lumens per unit area. One lambert is  $1 \text{ lumen cm}^{-2}$ , or  $1/\pi \text{ c cm}^{-2}$ . Likewise the foot-lambert, or apparent foot-candle, is  $1 \text{ lumen ft}^{-2}$ , or  $1/\pi \text{ c ft}^{-2}$ . The brightness of sources is commonly expressed in lamberts, candles per square millimeter, and candles per square centimeter.

In 1931 the International Commission on Illumination (ICI) (Optical Society of America, 1944b, 1953) established a tristimulus system of color specification involving three coordinates  $X$ ,  $Y$ , and  $Z$  for the three primary colors, red, green, and blue. All the luminosity was assigned arbitrarily to the  $Y$ , or green primary. The relative values of  $Y$ , designated as  $\bar{y}$ , vary from 0 to 1 and are the standard luminosity coefficients. The  $\bar{x}$  and  $\bar{z}$  coefficients are for the red and blue primaries and carry no luminosity. When plotted against wave length (Fig. 3-2), the  $\bar{y}$  function yields the spectral-sensitivity curve for photopic or daylight vision for the light-adapted cones of the eye of the Standard Observer, whose vision represents the average behavior of a group of normal individuals. The

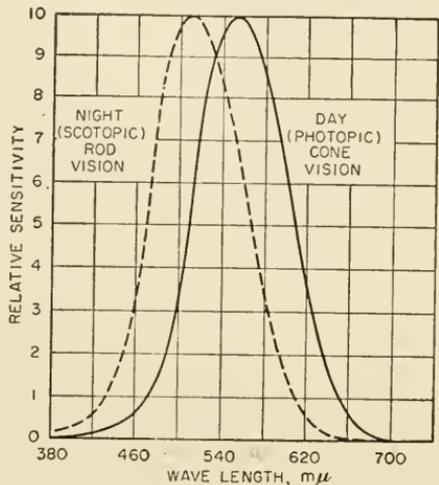


FIG. 3-2. Luminosity curves for the human eye. The solid curve for day vision is the ICI luminosity curve of  $\bar{y}$  coefficients. (From IES Handbook, 1952.)

approximate limits of the curve are 380 and 770  $m\mu$ , with a maximum at 555  $m\mu$ . The curve for rod vision (Illuminating Engineering Society, 1942; Weaver, 1937), as obtained with the dark-adapted eye, is shifted about 45  $m\mu$  to the shorter wave lengths, with a maximum at about 510  $m\mu$  (Fig. 3-2).

#### ENERGY EQUIVALENT OF LIGHT

The absolute luminous efficiency or absolute luminosity  $K$  for any source is given in lumens per watt of radiant energy, which is obtained by integrating the radiant flux for small wave-length intervals over the relative-luminosity curve. The maximum luminosity  $K_{\max}$  is the luminosity of monochromatic flux at 555  $m\mu$ , which has a value of about 650 lumens  $w^{-1}$  (Illuminating Engineering Society, 1942; Optical Society of America, 1944b, 1953). Values obtained by different observers vary from 625 to 680 lumens  $w^{-1}$ . The least mechanical equivalent of light is  $\frac{1}{650}$ , or 0.0015, w of monochromatic energy at 555  $m\mu$  and will produce 1 lumen of luminous flux. At 410 and 720  $m\mu$  the relative efficiency is 0.001, and therefore 1.5 w is required to produce 1 lumen at these wave lengths.

The absolute luminous efficiency of radiant energy should not be confused with electric-lamp efficiency, which is usually given by lamp manufacturers in lumens per watt of electrical-energy input and involves the efficiency of conversion of electrical energy to radiant energy as well as the absolute efficiency of the flux. The lumens per radiant watt  $K$  is always larger than the lumens per electrical watt; both are often referred to as "luminous efficiency."

The use of psychophysically derived quantities as the basis of radiometric evaluation is quite logical, providing the spectral limitations of the units are properly appreciated. For the animal or plant physiologist dealing with nonvisual photochemical problems, the spectral limitations imposed by use of the psychophysical units are often compensated for by the convenient availability of "light" meters calibrated in lux or foot-candles. The use of such units frequently yields more meaningful data than a total-energy measurement obtained with a nonselective detector such as a thermocouple, where the total unfiltered, or "white," energy of a source is measured. Where narrow spectral regions are employed, there is usually little justification for expressing intensities in any photometric type of unit.

#### PROPAGATION OF RADIANT ENERGY

##### INVERSE-SQUARE LAW

Since the propagation of radiant energy through space is rectilinear, the irradiance  $H$  produced by a point source of intensity  $J$  varies inversely as the square of the distance  $d$  from the source such that  $H = J/d^2$ .

For actual sources of finite size, the inverse-square law can be applied, without integration, with an error of less than 1 per cent when the largest dimension of the source or receiver is not more than one-tenth the distance between the two. This is a useful relation for predicting the irradiance obtainable at known distances from finite sources.

The opposite extreme from a point source is a uniformly distributed source, such as an overcast sky or a large bank of fluorescent lamps with closely spaced tubes. For infinite, uniformly distributed sources, the irradiance is independent of the distance. A parallel beam of radiant energy has similar properties and is approximated by a small source, such as a projection-lamp filament at the focus of a large parabolic mirror or plano-convex lens. Lamps mounted in reflectors used for general irradiation have properties that are intermediate between those of a point source and those of a distributed source.

#### LAMBERT COSINE LAW

The irradiance produced by a parallel beam of radiant flux is usually measured by determining the incident flux per unit of area normal (perpendicular) to the beam. However, for surfaces that are inclined from the normal by an angle of incidence  $\theta$ , the irradiance is decreased because the beam is distributed over a larger area, and  $H = (J/d^2) \cos \theta$ . In regard to sources, the cosine law states that the radiance or brightness of a black body is independent of the direction from which it is observed. For a finite plane source the intensity is independent of the angle at which it is observed, but the projected apparent area and total flux are proportional to the cosine of the angle of emittance. The angle of emittance  $\theta$  is included in the quantities radiance and brightness.

#### FRESNEL'S LAW OF REFLECTION

When a beam of radiant energy is incident to a smooth surface, specular reflection occurs such that the angle of incidence is equal to the angle of reflection, both measured from an axis normal to the surface. The proportion reflected from transparent surfaces is determined by the refractive indexes of the substances and the angle of incidence  $\theta$ , as given by Fresnel's law. For normal incidence ( $\theta = 0$ ) the proportion of the energy reflected  $R$  from a beam of intensity  $I$  is

$$R = \frac{I}{I_0} = \left( \frac{n_2 - n_1}{n_2 + n_1} \right)^2 \quad (3-5)$$

where  $I_0$  = intensity of incident beam,

$I$  = intensity of reflected beam,

$R$  = proportion reflected,

$n_2$  = refractive index of transparent substance, and

$n_1$  = refractive index of medium from which energy enters.

Thus, for radiant energy passing from air to water at normal incidence,  $n_1$  would be 1.0 and  $n_2$  would be approximately 1.3. The reflectance loss would be about 1.7 per cent. For window glass in air,  $n_2$  is approximately 1.5, and the reflectance is 4 per cent. For a pane of glass, the total reflectance loss at normal incidence for the air-glass and glass-air interfaces is about 8 per cent. At larger angles of incidence the losses are larger and may be calculated from data given in various physical tables and handbooks.

When the reflecting surface is highly irregular, diffuse reflection results. Diffuse reflection also occurs at surfaces made up of fine particles of transparent substances of large refractive indexes. Snow and deposits of fine crystals such as zinc oxide, titanium dioxide, and the other white paint "pigments" produce diffuse reflections, because the energy is refracted sharply at the crystal-air interfaces and internal reflection occurs, sending the beam back again in much the same manner as in a total-reflecting prism. This is the basis upon which white paint pigments are selected.

#### OPTICS OF IMAGE FORMATION

For many optical situations one can choose either a curved mirror or a lens. The same basic image formulas apply to both, and each has its own peculiar properties. Since the concave mirror and positive lens (plano-convex, double convex, or positive meniscus) are of most general use in simple condensing, collimating, and focusing systems, only these types will be discussed here. For further details reference is made to various texts in general physics and optics (Barrows, 1951; Habell and Cox, 1948).

*Image Formulas.* If the focal length is  $f$  (Fig. 3-3), the distance from the object to the surface of the mirror or the center of the lens is  $p$ , and the distance to the image is  $q$ , then the following equation applies to both mirrors and thin lenses:

$$1/f = 1/p + 1/q. \quad (3-6)$$

For real images the image is on the same side of the mirror as the object, whereas with the lens the object and image are on opposite sides. The distances  $p$  and  $q$  are known as the "conjugate" distances. When  $p$  is infinite,  $f = q$ , and the image is formed at the principal focus  $F$ . This is the condition for parallel rays incident on a positive lens or concave mirror. If  $p < f$ , i.e., if the object is inside the principal focus, no real image is formed; the rays appear to come from a virtual image (see Fig. 3-3), and  $q$  has a negative value.

The magnification of the image is given by the ratio of image to object distances:

$$M = q/p. \quad (3-7)$$

When the object and image distances are equal,  $M = 1$ , the image is the same size as the object, and  $p = q = 2f$ .

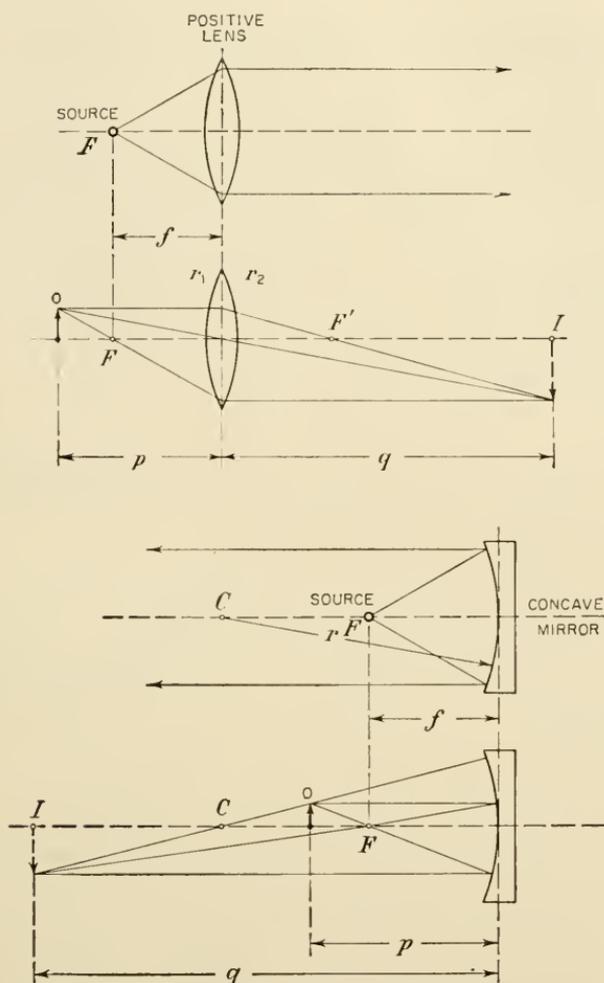


FIG. 3-3. Image formation in positive lenses and mirrors. The focus is at  $F$ , and the focal length is  $f$ ; the object is at  $O$ , and the object distance is  $p$ ; the image is at  $I$ , and the image distance is  $q$ . The radius of curvature is  $r$ .

For a mirror the principal focus is located on the axis halfway between the center of curvature  $C$  and the mirror surface (see Fig. 3-3); thus  $r = 2f$  for a mirror. The focal length of a thin lens in air is determined by the radii of curvature of the lens surfaces and the refractive index of the lens:

$$1/f = (n - 1)(1/r_1 + 1/r_2), \quad (3-8)$$

where  $n$  is the refractive index and  $r_1$  and  $r_2$  are the radii of curvature of the surfaces. The plano-convex lens has one surface that is a plane, i.e.,  $r_1$  is infinite, and the formula becomes

$$1/f = (n - 1)/r. \quad (3-9)$$

The radii are positive for convex surfaces and negative for concave surfaces.

*Image Defects.* The preceding equations are only approximately correct for spherical surfaces. Parallel rays are not brought to a sharp focus by either spherical mirrors or simple spherical lenses; this imperfection is known as "spherical aberration." Spherical aberration is avoided in concave mirrors by the use of a parabolic surface that has the property of bringing all rays parallel to the principal axis to a focus at one point  $F$ . The parabolic reflector is used in astronomical mirrors because the objects are at essentially infinite distance and the incident rays are parallel. They are also used in spectroscopy collimators for producing parallel flux and as focusing optics for conveying the parallel rays into the focal curve of the spectrum. The ellipse is the ideal curve for condensing mirrors where two conjugate foci are used, since the ellipse causes all rays from one focus to be imaged at the other.

Spherical aberration is corrected in lenses by the proper choice of radii of curvature and the use of aspherical surfaces. In plano-convex lenses the spherical aberration is at a minimum when the parallel rays are incident to the curved surface (Fig. 3-23a). For this reason plano-convex lenses are often used in condensing systems, with the convex surfaces facing one another.

A second image-forming defect of both mirrors and simple lenses is astigmatism, which arises when an image is formed by rays that originate off the principal axis. The rays from a point off the axis are brought to a line focus at one point and a second line focus at another point; the line foci are perpendicular to one another. Between the two line foci is a region of least confusion, which is often taken as the focal point.

The third image defect, chromatic aberration, is present in lenses but not in mirrors. It arises from the variation in refractive index of the lens material with wave length. If parallel rays of the visible spectrum are incident on a lens, the blue rays will be brought to a focus closer to the lens than the red rays (Fig. 3-3); i.e., the rays are dispersed into a short spectrum along the lens axis. Chromatic aberration may be eliminated partially by the use of compound lenses known as "achromats," having components of different dispersions such that the dispersive effects of the two components nullify one another at two selected wave lengths. For the ultraviolet, achromats may be made from quartz-fluorite combinations.

One of the principal advantages of mirrors over lenses is the lack of chromatic aberration. Spectroscopes with mirror optics do not require the extreme tilting of the photographic plate necessary in lens instruments, and the focal adjustments are the same for all wave lengths.

*Aperture.* The brightness or intensity of the image produced by parallel rays from a distant object incident to a lens or mirror, as well as the flux-gathering power of a condensing system, is primarily a function of the diameter and focal length. This determines the solid angle  $\omega$  subtended by the bundle of rays converging upon an image at the focus or diverging from a source at the focus. It is evident from the lens formula that, as the focal length is decreased and the solid angle is increased, the image becomes smaller and more intense. The image intensity is proportional to the subtended solid angle, which is the angular aperture or aperture of the lens or mirror. Since, in radian measure,  $\omega = A/f^2$ , where  $A$  is the area on a sphere of radius  $f$ , the aperture is given approximately as

$$\omega = 4\pi(d/f)^2, \quad (3-10)$$

where  $d$  is the diameter of the optical element.

The  $f/$  number for camera lenses is the ratio  $f/d$ , which is also known as the "aperture ratio." The exposure required for a photographic lens is proportional to the square of the  $f/$  number, or aperture ratio. Conversely, the speed of the lens is inversely proportional to the square of the aperture ratio. The linear aperture is equal to the effective diameter of circular optical elements, but for a rectangular element such as a prism, there may be two linear apertures referring to the effective height and width. The aperture area is the effective area of the optical element.

#### ABSORPTION OF RADIANT ENERGY

When radiant energy traverses matter, it is attenuated to a degree depending upon the probability that a photon will be captured by an atom or molecule in its path and converted into some other form of energy. In the far infrared the quantum energy is small, and the consequences of absorption can result only in an increase in the rotational energy of the molecules and the immediate degradation of the photon energy to translational or heat energy. In the near infrared both the vibrational and rotational energy levels of the capturing molecules may be increased. As the photon energy increases in passing to shorter wave lengths, the site of interaction moves deeper into the atomic structure. In the visible and near ultraviolet the interaction concerns mainly the outer valence electrons, and valence bonds may be altered, thus bringing about a photochemical reaction. In the far ultraviolet the energy may be sufficient to eject the outer electron completely from its atom and produce ionization. The X-ray photon can interact with the inner electrons

of the atom, and with  $\gamma$  rays and other nuclear forms of radiant energy the atomic nuclei may be the site of interaction, although all the other forms of interaction may also be present.

#### BOUGUER-BEER LAW

There are two generalized laws governing radiant-energy absorption which are mathematical statements as to the probability of capture of a photon by the absorbing molecules or atoms in the path of the beam in relation to the thickness and concentration of the absorbing substance. It was shown by Bouguer (Brode, 1949; Gibson, 1949; Mellon, 1950) (frequently attributed to Lambert) that the rate of decrease of intensity  $I$  is proportional to the thickness  $x$  and that  $-dI/I = \mu' dx$ , where  $\mu'$  is a constant that is an intrinsic property of the absorbing molecules and is a function of wave length. Integrating between the limits of incident intensity  $I_0$  and transmitted intensity  $I$  for a finite thickness  $b$  gives the exponential expression for Bouguer's law,  $I = I_0 e^{-\mu' b}$ , where  $e$  is the natural-logarithm base, 2.718. Beer derived a similar relation for the absorption of solutes in relation to concentration. When combined, the two relations yield the Bouguer-Beer law of absorption,

$$I = I_0 e^{-\mu c b} \quad (3-11)$$

and

$$-\ln I/I_0 = -\ln T = \mu c b,$$

or

$$\ln I_0/I = \ln 1/T = \mu c b,$$

where  $T$  = transmittance or ratio of transmitted to incident flux,

$c$  = solute concentration,

$b$  = internal cell thickness, and

$\mu$  = absorption coefficient (Brode, 1949).

This relation is shown in Fig. 3-4. As  $\mu$  increases, the absorption increases and the transmittance decreases.

#### ABSORPTION AND TRANSMISSION

Since the measurement of absorption involves only the ratios of the incident and transmitted beams, any convenient system of comparison may be used. Consequently the notation  $I$  ( $J$  in German literature) has been rather generally accepted as the symbol for the relative beam intensities in terms of radiant energy or radiant flux (*ibid.*). For the other terms and notations there has been much less agreement, but as the result of a study of the respective literatures, Brode (1949) and Gibson (1949) have developed nomenclatures that are consistent and precise. The nomenclature of Gibson will be used here. It is similar to that of Brode but is somewhat more extensive.

In the determination of spectral absorption one is usually concerned either with the total absorption of a sample in which all constituents are summated in the result or with the absorption of one group of constituents to the exclusion of others. An example of the first type is the measurement of the transmission of a glass color filter. The second is typical of the chemical spectrophotometry of solutions in which a comparison cell of solvent cancels out cell and solvent absorption. For the two classes of notations, Gibson has proposed different suffixes, *-ance* for the summated

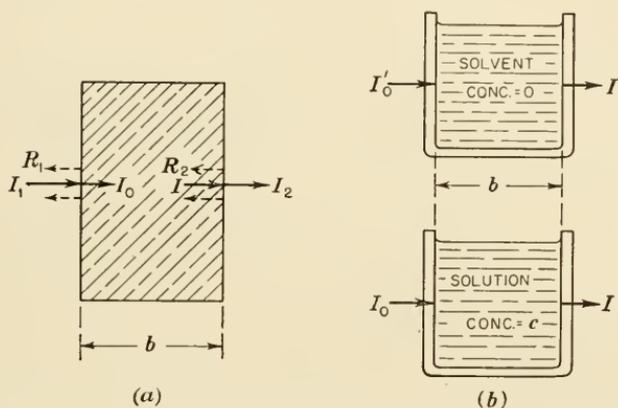


FIG. 3-4. Diagram of the quantities involved in the measurement of transmission and absorption.

system treated as a single component and *-ancy* for the multicomponent system.

The following assumptions are made in precise spectrophotometry: (1) the sample is homogeneous and isotropic, so as not to polarize, deviate, or scatter the beam from a rectilinear path; (2) both surfaces are smooth, parallel, and normal to the beam; (3) the beam divergence is sufficiently small so that there is negligible variation in path length over the beam cross section; (4) the radiant energy is unpolarized; and (5) the energy is monochromatic. The maximum permissible spectral band width as required in item 5 is determined by the sharpness of the absorption bands of the sample. These factors are discussed in detail by Gibson (1949), Mellon (1950), and Brode (1949).

*Single-component Systems.* These terms relate to the transmission of monochromatic radiant energy by homogeneous, isotropic, nonmetallic media. They are applied to the complete sample of a single gas, liquid, or solid or to a complex system such as a multiple-layer filter. In the case of liquids and gases, absorption due to the vessel may be deducted from the over-all determination by the use of a short comparison cell, as diagramed in Fig. 3-4a.

$$\begin{aligned}
 T &= I_2/I_1 = \text{transmittance of sample (includes reflection losses).} \\
 100T &= \% T = \text{percentage of transmittance.} \\
 T_i &= I/I_0 = \text{internal transmittance of sample, corrected for reflection losses, window absorption at interfaces, and cell windows.} \\
 A_i &= -\log T_i = \log 1/T_i = \log I_0/I = \text{internal absorbance of sample.} \\
 a_i &= A_i/b = \text{absorbance index, or absorbance per unit thickness.} \\
 D &= \log I_2/I_1 = \text{density, an absorbance term applied to nonspectral determinations such as the blackening of photographic emulsions.} \\
 I_a &= I_0 - I = \text{absorbed flux.} \\
 \alpha &= 1 - T_1 = \text{absorptance, or proportion absorbed by sample. This quantity is of special interest in analysis of action spectra, since } \alpha \text{ is approximately equal to } \mu \text{ at low values of absorptance.}
 \end{aligned}
 \tag{3-12}$$

*Multicomponent Systems.* These terms relate to homogeneous mixtures such as solutions in which the absorption of one constituent, usually the solvent, is canceled out of the measurement by a comparison cell of that constituent. Following the notation of Fig. 3-4b,

$$\begin{aligned}
 T_{\text{solv}} &= I'/I'_0 = \text{internal transmittance of comparison cell containing solvent or solution lacking the unknown constituent; and} \\
 T_{\text{soln}} &= I/I_0 = \text{internal transmittance of cell containing complete solution.}
 \end{aligned}$$

If identical, matching absorption cells are used, reflection and window losses are nearly the same, and only the internal transmittance need be considered. When the solute contributes appreciably to the refractive index, however, correction for differences in reflection at the solution-glass interfaces may be required. Then

$$\begin{aligned}
 T_s &= T_{\text{soln}}/T_{\text{solv}} = \text{transmittancy of unknown constituent of sample.} \\
 100T_s &= \% T_s = \text{percentage transmittancy.} \\
 A_s &= -\log T_s = \log 1/T_s = abc = \text{absorbancy.} \\
 a_s &= A_s/bc = \text{absorbancy index (absorptivity } K\text{).}
 \end{aligned}
 \tag{3-13}$$

If  $c$  is in grams per liter and  $b$  is in centimeters, the unit is liters per gram-centimeter.

$a_m$  = molar absorbancy index (molar absorptivity).

If  $c$  is in moles per liter and  $b$  is in centimeters, the unit is liters per mole-centimeter.

## REFLECTION

Reflection measurements are made in a manner similar to transmission measurements. Total reflectance is defined as the ratio of reflected intensity  $I_R$  to incident intensity  $I_0$  without regard to direction and includes both the specularly and diffusely reflected flux; thus  $I_R/I_0$  = total reflectance. Specular reflectance involves only that flux which emerges from the sample at an angle equal to the angle of incidence and does not include scattered flux.

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## 2. SOURCES OF RADIANT ENERGY

Sources for the visible and adjacent spectral regions may be divided arbitrarily into three general classes: the thermal radiator, the electrical discharge or electron-excited source, and the fluorescent lamp. The thermal, or complete, radiators include the sun and the incandescent lamps. The spectral energy distribution is continuous, with a single maximum that is in the near infrared for most artificial sources. The electrical discharge sources include arcs and discharges in metallic vapors and inert gases. The spectrum usually consists of lines characteristic of the elements present in the discharge, although the line spectrum may be superposed on a continuous background of thermal radiation from hot rare gases and incandescent electrodes. In the fluorescent lamp, ultraviolet energy from a low-pressure discharge is absorbed by a phosphor coating on the inside of the lamp and then, by fluorescence, reemitted at longer wave lengths.

Although in biological research one is seldom interested directly in the design or construction of lamp sources, basic data on fundamental elements of design and electrical and radiation characteristics can be helpful in the selection of the proper lamp for a research application. It is toward this objective that the following physical and engineering data are given. For discussions of the characteristics and application of sources of all types, reference is made to the general articles by Aldington (1945), American Society for Testing Materials (1946), Barnes *et al.* (1939), Dushman (1937), Greider (1931); to the books by Bourne (1948), Forsythe (1937), Harrison *et al.* (1948), Illuminating Engineering Society (1952), Koller (1952), Macbeth and Nickerson (1949), Strong (1943); and to the manufacturer's pamphlet by Weitz (1950).

### THERMAL SOURCES

#### GENERAL CHARACTERISTICS

*Black-body Radiator.* The spectral energy distribution of most incandescent thermal sources can be approximated closely by the complete radiator (variously referred to as "perfect," "black-body," "ideal," or "Planckian") at a hypothetical temperature. The radiation laws of such a complete thermal radiator are completely derived from thermodynamic and quantum theories. The "complete," or "black-body," radiator is so called because it is assumed to be a complete absorber of radiant energy in all spectral regions and neither transmits nor reflects radiant energy. Such a hypothetical body radiates more power (flux) at any given temperature than any other body at the same temperature, provided that the radiant flux is due solely to thermal collision. The spectral energy distribution and total power radiated by a complete radiator are a function only of its temperature.

A uniformly heated opaque enclosure or cavity, containing a small opening for sampling the radiant flux, is a nearly perfect complete radiator source. The character of the escaping energy is a function only of the temperature of the enclosure and is completely independent of its composition. The flux, radiated by the walls, encounters internal multiple reflections, and all the flux is eventually absorbed by the walls except for the small sample that escapes through the opening. The noble metals provide known melting points with which to control the temperature of the cavity. Experimental complete radiators are used principally as radiation standards. Forsythe (1937) describes the construction of several standard complete radiator sources.

It was shown by Kirchoff that the capacity of a substance to emit radiant energy is proportional to its ability to absorb that same energy. Thus highly absorbing "black" materials such as carbon are more efficient radiators than bright polished metals such as aluminum and tungsten. If  $W$  is the radiant emittance of a body,  $\alpha$  the absorptivity for radiant energy of the same spectral distribution, and  $W_b$  the radiant emittance of a complete radiator, then  $W = \alpha W_b$ . For all actual materials  $\alpha$  is less than 1, and the power radiated at any temperature is always less than that of a complete radiator.

The relation between the total flux per unit area  $W$  and the absolute temperature  $T$  for a complete radiator is given by the Stefan-Boltzmann law,

$$W = \sigma T^4, \quad (3-14)$$

where  $\sigma$  is a constant having the value  $5.672 \times 10^{-12} \text{ w cm}^{-2} \text{ deg}^{-4}$  (DuMond and Cohen, 1948; Illuminating Engineering Society, 1952). Spectral-energy-distribution curves for a complete radiator at various temperatures plotted on a logarithm scale of intensity are given in Fig. 3-5. As the temperature increases, the total area under the curve  $W$  increases rapidly as the fourth power of the temperature, and the wave

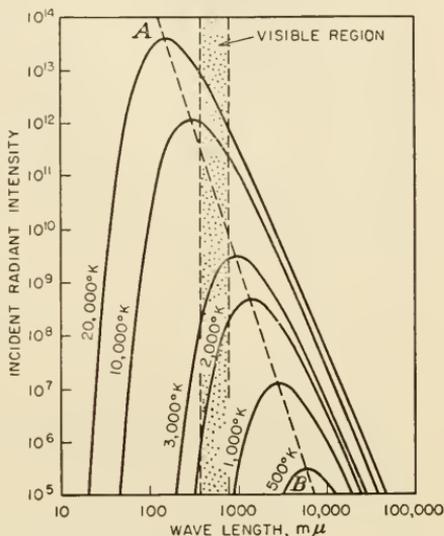


FIG. 3-5. Spectral emission of a black-body radiator at various temperatures plotted on a logarithm scale of wave lengths. As the temperature increases, the wave length of maximum emission  $\lambda_m$  along the line A-B shifts to the shorter wave lengths, as predicted by the Wien law. (From IES Handbook, 1952.)

length of maximum emittance  $\lambda_m$  shifts to shorter wave lengths in accordance with the Wien displacement law,

$$\lambda_m T = b, \quad (3-15)$$

where  $\lambda_m$  is in millimicrons,  $T$  is in degrees Kelvin, and  $b$  is a constant,  $2.896 \times 10^6$ . At a temperature of  $2896^\circ\text{K}$ ,  $\lambda_m$  is  $1000 \text{ m}\mu$ . At  $5782^\circ\text{K}$ , about the value of the surface temperature of the sun,  $\lambda_m$  is  $500 \text{ m}\mu$ .

Planck was the first to develop a radiation law that precisely described all the experimental facts regarding the spectral energy distribution of complete radiators. In deriving his law, Planck broke away from classical concepts of energy as a continuum and introduced the concept that radiant energy is emitted and absorbed discontinuously in discrete units that are proportional to the frequency:  $E = h\nu$ . The spectral radiant emittance of a complete radiator for small intervals of wave length is given by Planck's formula,

$$W_\lambda = c_1 \lambda^{-5} (e^{c_2/\lambda T} - 1)^{-1} \quad (3-16)$$

where  $W_\lambda$  = spectral radiant emittance,  $\text{w cm}^{-2}$  per micron of wave-length interval,

$e$  = Napierian logarithm base, 2.718,

$A$  = area of the source,  $\text{cm}^2$ ,

$c_1$  = first radiation constant, 37,350,

$c_2$  = second radiation constant, 14,380,

$\lambda$  = wave length,  $\mu$ , and

$T$  = temperature,  $^\circ\text{K}$ .

The Planck formula is difficult to calculate, and for most practical purposes more simplified approximate formulas are used. Tables giving the radiant power per unit wave length have been computed by various authors (Fowle, 1929; Frehafer and Snow, 1925; Holladay, 1928; Moon, 1937; Skogland, 1929).

*Selective Radiator.* The Planckian radiation laws may be applied to non-Planckian, or selective, radiators by employing the emissivity factor  $\epsilon$ . The Stefan-Boltzmann law is then modified as follows:

$$W = \epsilon_t \sigma T^4, \quad (3-17)$$

where  $\epsilon_t$  is the total emissivity. It is 1 for a complete radiator and less than 1 for all actual substances. The absorptivity  $\alpha$  from Kirchoff's law and total emissivity can be shown to be equal; therefore the total emissivity of any radiator is equal to its absorptivity for the flux emitted by a complete radiator operating at the same temperature (Forsythe, 1937). The total emissivity is a function of the composition of the radiator and its temperature. The spectral emissivity  $\epsilon_\lambda$  is a variable that is a function of composition, temperature, and wave length.

Since the complete radiator is a highly reproducible source whose radi-

ation properties are a unique function of temperature and may be calculated with precision, it has become the practice to specify incomplete or selective radiators by that temperature at which a complete radiator has similar radiation characteristics. Since the spectral emissivity is a function of both temperature and wave length, the comparison can be made only on the basis of one of the three criteria: (1) radiance, (2) brightness, or (3) color, or spectral energy distribution in the visible region.

The radiation temperature of a source is that temperature at which a complete radiator produces the same total radiant flux per unit area (radiant emittance) as the selective radiator. Since complete radiators are the most efficient, the radiation temperature is always less than the true temperature. The brightness temperature is that temperature at which a complete radiator produces the same brightness (luminous flux per unit area) as the selective radiator. Optical pyrometers for the remote measurement of the temperature of incandescent objects, such as the interior of furnaces, measure brightness temperature. The color temperature of a source is the temperature at which a complete radiator produces a chromaticity or color match with the source. The color temperature may be higher than the actual temperature of the selective radiator, because many metals, including tungsten, emit radiant flux at a given temperature with the wave-length maximum shifted toward the shorter wave lengths as compared with a complete radiator at the same temperature. Color temperatures of tungsten-filament lamps are determined by various methods, the more important of which have been reviewed by Harding (1950). One method involves the determination of the ratio of blue to red flux, thus determining the average slope of the emission curve in the visible region.

*Luminous Efficiency.* The luminous efficiency of the radiant energy of a source is probably the most convenient means of expressing the proportion of the total spectral energy within the visible spectrum. It must be recognized, however, that the term involves the evaluation of the energy over the luminosity curve (Fig. 3-2). Luminous efficiency may be expressed in various units, but those most commonly used are lumens per watt (radiated watts, not electrical) and lux or foot-candles per calorie per minute per square centimeter. The foot-candle per cal  $\text{min}^{-1} \text{cm}^{-2}$ , also referred to as the illumination equivalent of 1 cal  $\text{min}^{-1} \text{cm}^{-2}$ , has been used by the Weather Bureau for the evaluation of solar energy (Kimball, 1924).

The upper limit of irradiance which plant and animal tissues can tolerate is often determined by the heating effect of the absorbed energy. Therefore, in studies of vision, photosynthesis, and other mechanisms involving only the visible, the infrared is an undesirable component that may represent the largest proportion of the total energy. In direct noon summer sunlight in the tropical and temperate zones, plants and ani-

imals are exposed to a maximum irradiance of the order of  $1000 \text{ w m}^{-2}$ , or  $1.5 \text{ cal min}^{-1} \text{ cm}^{-2}$ . This approaches the maximum flux tolerated by most organisms in an air environment. Solar radiant flux has a luminous efficiency of about  $100 \text{ lumens w}^{-1}$ , or  $6500 \text{ ft-c per cal min}^{-1} \text{ cm}^{-2}$ . The maximum solar visible irradiance is then about  $10,000 \text{ ft-c}$ . Incandescent-lamp energy of  $2000 \text{ ft-c}$  at  $20 \text{ lumens w}^{-1}$  will produce the same total irradiance and nearly the same heating effect as noon sunlight. If water is used to absorb the infrared from an incandescent source, the luminous efficiency of the radiated flux can be increased close to that of solar flux, and the total amount of visible flux that can be tolerated is increased by a factor of 5 (Gordon, 1930).

*Radiance and Brightness.* The intensity of a source may be specified in terms of radiance, which is power per unit solid angle and area; radiant emittance, power per unit area; or radiant intensity, power per unit solid angle. The photometric analogue of radiance is brightness, candles per unit area, which is equivalent to lumens per unit solid angle and area. These quantities are available for many commercial sources and serve as useful guides in comparing the total and visible intensity of sources.

Sources of high intensity must be selected when it is necessary to pass the maximum flux through an optical system such as a monochromator. For the irradiation of large areas where optical systems are not involved, low-intensity or low-brightness sources may be used. Sources employed for the general irradiation of plant material or for general lighting may be of this type. The fluorescent lamp is a typical source of low brightness, and the carbon arc, of high brightness.

#### THE SUN

The sun is an incandescent stellar body having a gaseous outer envelope that is maintained at about  $6000^\circ\text{K}$  by nuclear reactions occurring in the interior. The spectrum at the earth's surface appears as that of a thermal radiator modified by the emission and absorption spectra of the lighter elements in the sun's envelope and absorption by the various components of the earth's atmosphere. The sun is unsurpassed as an economical source of visible energy, and the high irradiances prevailing during clear weather are difficult to reproduce with artificial sources. The nature of the sun and its radiation (Menzel, 1949; Nicolet, 1943; Roberts, 1952) and the manner in which solar irradiance changes as a function of time and place over the earth's surface have been discussed extensively by many authors (Benford, 1947a,b, 1948a,b; Hand, 1937, 1941, 1950; Kimball, 1924; Moon, 1940).

*Solar Constant.* Measurements made on the intensity of solar energy after it has passed through various thicknesses of the earth's atmosphere have made it possible to calculate the solar irradiance at normal incidence just outside the earth's atmosphere at the mean solar distance of the

earth from the sun. This quantity, known as the "solar constant," has a value of  $1.94 \text{ cal min}^{-1} \text{ cm}^{-2}$ , or  $1350 \text{ w m}^{-2}$  (Abbott, 1952; Moon, 1940). The solar constant changes  $\pm 3.5$  per cent from the mean owing to variations in distance from the sun to the earth as the earth moves through its orbit. In addition, the solar constant shifts  $\pm 2$  per cent in an irregular manner owing to deviations in the activity of the sun itself.

*Transmission of the Atmosphere.* Large variations in solar irradiance at the earth's surface result from changes in scattering and absorption by the earth's atmosphere. The calculation of spectral-solar-radiation curves and the factors responsible for energy losses in the atmosphere have been extensively reviewed by Moon (1940) and Benford (1948b). Molecules of the atmospheric gases and fine particles of dust scatter much of the incoming energy. Since the dimensions of these particles are small compared with the wave lengths of visible energy, the intensity of scattering is inversely proportional to the fourth power of the wave length in accordance with Rayleigh's law, which states that the scattering is inversely proportional to the fourth power of the wave length. Since the shorter blue wave lengths are scattered more than the longer red wave lengths, a clear sky appears blue. During very cloudy weather most of the incoming energy may be reflected back into space by thick layers of small particles of liquid water or ice, which compose the clouds. These particles are relatively large as compared with the visible wave lengths, and therefore there is little selective scattering; consequently clouds usually appear white.

Most of the absorption of solar radiant energy by the atmosphere in the near infrared is due to water vapor and carbon dioxide, and in the middle ultraviolet, to ozone; the atmosphere of a clear sky is quite transparent to the visible and near ultraviolet. The transmittance of an air mass of 1, which occurs only when the sun is overhead (solar angle  $90^\circ$ ), is about 80 per cent throughout most of the visible. Water-vapor bands strongly attenuate the near infrared from 720 to 2300  $\mu$ ; the infrared is absorbed almost completely by atmospheric water vapor and carbon dioxide beyond about 2300  $\mu$ . The transmission of the atmosphere for a heavily overcast midday sky may fall to a few per cent.

The ultraviolet limit of terrestrial solar energy is at about 300  $\mu$ , limited principally by the absorption of ozone in the ionosphere. The densest portion of the ozone layer varies in altitude between 22 and 25 km. It begins to absorb at about 320  $\mu$  and becomes practically opaque at 290  $\mu$ , as recorded by Stair (1951).

*Air Mass.* The equivalent number of atmospheric thicknesses traversed by the sun's rays is approximately proportional to the cosecant of the solar angle as measured by a tangent to the earth's surface. As the sun appears to move from the zenith position, or  $90^\circ$  solar angle, where the air mass is 1, the air mass increases slowly at first and then rapidly

as the sun approaches the horizon until at  $5^\circ$  it is 10.4 and at  $1^\circ$  it is 27. The air mass at small solar angles is larger than would be derived from purely geometrical considerations, because the variation in density of the atmosphere causes the rays to be refracted toward the horizon. This accounts for the rapid attenuation at sundown and the orange-to-red color when the sun is close to the horizon. In passing close to the earth's

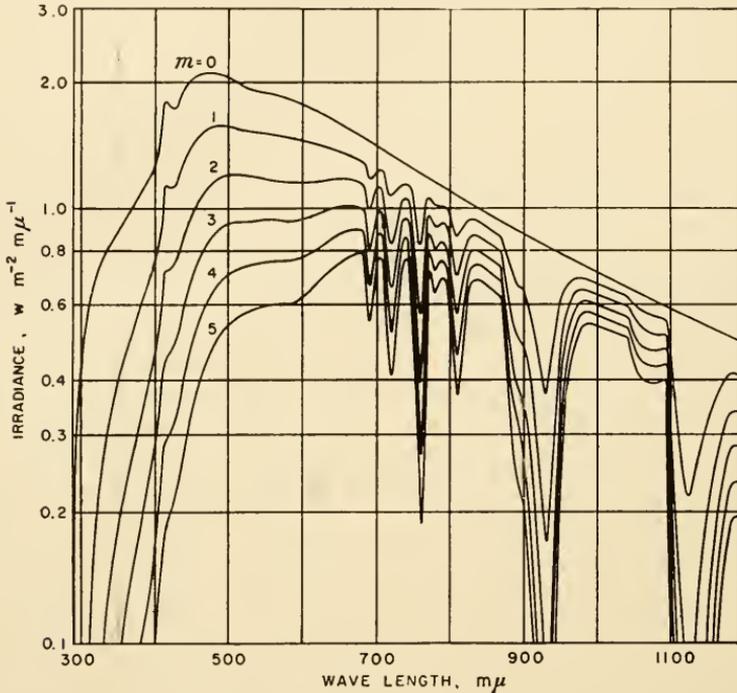


FIG. 3-6. Spectral energy distribution of solar energy for air masses from 0 to 5. (From Moon, 1940.)

surface, the rays traverse dust-laden air, which, together with the other constituents, severely scatters the shorter wave lengths.

*Spectrum.* Figure 3-6 gives the spectral energy distribution of solar radiant energy at air masses from 0 to 5, as summarized by Moon (1940). The curve for zero air mass corresponds to the spectral energy distribution outside the atmosphere. It will be noted that, as the air mass increases, the wave length of maximum energy  $\lambda_m$  shifts from about  $470 \text{ m}\mu$ , at an air mass of 1, to  $650 \text{ m}\mu$ , at an air mass of 5. The sky-radiation curve has a maximum in the blue and falls rapidly with increasing wave length (Abbott *et al.*, 1923; Taylor and Kerr, 1941). These data are summarized in Table 3-8. Most of the energy is within the range  $700\text{--}1100 \text{ m}\mu$ ; relatively little energy, less than 10 per cent, is in the infrared beyond  $1500 \text{ m}\mu$ . The color temperature of the solar disk varies between  $6000^\circ$  and  $6800^\circ\text{K}$  for a clear sky. The average for the

sun and sky in the temperate zone in the vicinity of a large city varies between 5000° and 6500°K (Taylor and Kerr, 1941).

*Intensity.* The solar disk has an apparent intensity, when observed at the earth's surface, of 50 w m<sup>-2</sup> and a brightness of 1600 c mm<sup>-2</sup>. Few artificial sources can equal the intensity of the sun. Terrestrial solar irradiance is very variable and is determined primarily by solar

TABLE 3-8. RELATIVE SOLAR IRRADIANCE, LUMINOUS EFFICIENCY, AND COLOR TEMPERATURE AT SEA LEVEL FOR VARIOUS AIR-MASS VALUES (Adapted from Moon, 1940, Table IV.)

Air mass.....	0	1	2	3	4	5
Solar angle.....	.....	90°	30°	19.3°	14.3°	11.3°
Wave length, mμ	Percentage of total irradiance					
290-400	7	4	3	2	1	1
400-700	41	46	45	43	41	38
700-1100	28	33	36	38	40	42
1100-1500	12	10	9	9	9	9
1500-∞	12	7	7	8	9	10
Total.....	100	100	100	100	100	100
Total irradiance <sup>a</sup> w m <sup>-2</sup> .....	1320 <sup>b</sup>	930	740	610	510	430
lumens w <sup>-2</sup> .....	93	105	106	103	98	93
ft-c g-cal <sup>-1</sup> min <sup>-1</sup> cm <sup>-2</sup> ..	6000	6800	6850	6650	6300	6000
Color temperature, °K....	6200	5500	5100	4700	4300	4100

<sup>a</sup> Multiply by 100 for microwatts per square centimeter and by 0.0014 for calories per minute per square centimeter.

<sup>b</sup> Value of solar constant.

angle and climatic factors. Benford (1947b, 1948a,b) and Hand (1950) have plotted the manner in which the irradiance varies with solar angle in relation to a normal and a horizontal plane. Kimball (1924; Kimball and Hand, 1936) and Hand (1937, 1941) of the U.S. Weather Bureau have reported detailed summaries of the daily and seasonal variations in solar irradiance at many locations in the United States, including Alaska and Puerto Rico; Crabb (1950) has summarized the data for the state of Michigan.

The highest total irradiance attained at sea level in the temperate zone is about 1.5 cal min<sup>-1</sup> cm<sup>-2</sup>, or 1000 w m<sup>-2</sup>, and a visible irradiance of about 10,000 ft-c. The values at high elevations and in dry, dust-free climates occasionally may be as much as 20 per cent higher. The average daily total of energy in the United States during the summer months

varies between 400 and 600 cal cm<sup>-2</sup>, depending upon climatic conditions. For the entire year in the United States the average varies with locality from 250 to 450 cal cm<sup>-2</sup>. Of this energy, only about 40 per cent is within the range 400-700 m $\mu$  (Table 3-8). For an average cloudy sky, Kimball and Hand (1936) have shown that the visible irradiance varies from a maximum of about 1500 ft-c to a minimum of about 500 ft-c during the middle of the day.

One striking feature of solar-irradiance data is the slight difference shown in maximum intensity and spectral composition between clear

TABLE 3-9. TIME FROM SUNRISE TO SUNSET ON THE TWENTY-FIRST DAY OF EACH MONTH FOR EACH 10° OF LATITUDE (Eckert and Clemence, 1946.)

Latitude, deg	0	10	20	30	40	50	60	70
Month	Time, hr and min							
January.....	12-07	11-39	11-07	10-33	9-49	8-48	7-08	2-28
February.....	12-07	11-52	11-36	11-18	10-58	10-28	9-44	8-17
March.....	12-07	12-07	12-07	12-09	12-11	12-13	12-18	12-25
April.....	12-07	12-24	12-42	13-04	13-30	14-07	15-05	17-07
May.....	12-07	12-37	13-09	13-47	14-34	15-40	17-35	L <sup>a</sup>
June.....	12-07	12-43	13-21	14-05	15-01	16-23	18-53	L <sup>a</sup>
July.....	12-07	12-37	13-10	13-48	14-36	15-44	17-41	L <sup>a</sup>
August.....	12-07	12-24	12-42	13-04	13-32	14-09	15-09	17-13
September.....	12-07	12-08	12-08	12-10	12-13	12-17	12-23	12-34
October.....	12-07	11-51	11-35	11-17	10-55	10-26	9-41	8-12
November.....	12-07	11-38	11-07	10-32	9-48	8-47	7-07	2-23
December.....	12-07	11-32	10-55	10-12	9-20	8-04	5-52	D <sup>a</sup>

<sup>a</sup> L, continuous daylight; D, continuous darkness.

summer days at any latitude within the tropical and temperate zones. The important differences are chiefly due to the greater proportion of clear days in one region as contrasted with another. Ultraviolet studies (Coblentz and Stair, 1944) have resulted in similar conclusions. The middle-ultraviolet irradiance, about 320 m $\mu$ , may be nearly as great on a clear day at latitude 62° N (Alaska) as at 39° N (Washington). In the tropics the ultraviolet intensity may be 20 per cent higher than in the temperate zone for clear days and the same solar angle, owing to lower ozone concentration. Weather data on the proportion of overcast skies and information on atmospheric pollution in the vicinity of large cities can be used as an approximate means of comparing the relative amounts of solar energy to be expected at any two locations.

*Day Length.* The flowering of many plants and the sexual behavior of certain animals are influenced by the length of the daylight period. In the northern hemisphere the longest days occur on June 21 and the short-

est on Dec. 21. The relation is reversed for the southern hemisphere. In Table 3-9 are given the day lengths for the twenty-first day of each month for each  $10^\circ$  of latitude from the equator north to the seventieth parallel. The day length was taken as the time from the first appearance of the upper edge of the solar disk in the morning to its last appearance in the evening. It takes into account the refraction of the sun's rays by the atmosphere (List, 1951; U.S. Naval Observatory, 1946).

#### INCANDESCENT TUNGSTEN LAMP

The tungsten-filament incandescent lamp is undoubtedly the most versatile and generally useful of all artificial sources for the visible and near infrared. It has highly stable electrical and radiation characteristics and ordinarily requires no special auxiliary electrical equipment. It is manufactured commercially in a wide variety of bulb and filament sizes, shapes, and power ratings ranging from the small 0.17-w surgical lamp to the 10-kw airport floodlight lamp (Weitz, 1950).

*Radiation Properties of Tungsten.* The total and spectral characteristics of the radiation emitted by a tungsten lamp are wholly dependent upon the thermal-radiation properties of incandescent tungsten metal within the limits imposed by the envelope. The electrical power required serves only to heat the filament; it contributes nothing intrinsically to the radiation. An incandescent tungsten filament is a selective thermal radiator whose emission, total and spectral, is primarily a function of temperature and secondarily one of filament configuration.

Tungsten has a melting point of  $3653^\circ\text{K}$  ( $3380^\circ\text{C}$ ), the highest of any known metal. In commercial lamps it is operated as a filament to as high as  $3350^\circ\text{K}$ , which is within  $300^\circ$  of its melting point. The radiation properties of tungsten from  $1500^\circ\text{K}$  to its melting point are given in Table 3-10, and the spectral energy distribution for a series of filament temperatures in Fig. 3-7 (Barnes and Forsythe, 1936a; Jones and Langmuir, 1927a,b,e; Forsythe and Worthing, 1925). The emissivity increases with temperature, and total emissivity varies between 0.19 at  $1500^\circ\text{K}$  and 0.35 at  $3500^\circ\text{K}$  (Coblentz *et al.*, 1926; Coblentz and Stair, 1936, 1944; Conn, 1951). The color temperature of tungsten is slightly higher than the true temperature because of its selective emission in the shorter wave lengths, a fact that contributes materially to the high luminous efficiency of tungsten filaments.

The spectral energy distribution of a uniformly heated straight round filament may be obtained from the product of the spectral emissivity  $\epsilon_\lambda$  and the spectral radiant intensity for a complete radiator at that temperature. The spectral emissivity is a function of both temperature and wave length (Table 3-10 and Fig. 3-7). The emissivity rises slowly with decreasing wave length to a maximum in the vicinity of  $300\text{ m}\mu$ , after which it falls sharply (Forsythe and Adams, 1945; Ornstein, 1936).

Because of the low emissivity beyond  $300\text{ m}\mu$  and the inherently weak ultraviolet emission of thermal radiators, the tungsten-filament lamp is an inefficient ultraviolet source. Since any cavity in which the radiant energy undergoes multiple internal reflections approaches a Planckian radiator in emissivity, a coiled filament is intermediate in spectral emissivity between that of a straight tungsten wire and a complete radiator.

TABLE 3-10. RADIATION PROPERTIES OF TUNGSTEN  
(Data adapted from Jones and Langmuir, 1927a, and Forsythe and Worthing, 1925.)

Filament temperature, °K	Color temperature, °K	Radiation temperature, °K	Total emissivity	Brightness, $\text{cm}^{-2}$	Luminous efficiency, lumens per radiated watt	Radiant emittance, $\text{w cm}^{-2}$
1500	1520	990	0.19	0.3	0.2	6
1800	1830	1250	0.24	5.0	1.2	14
2000	2030	1430	0.26	20	2.8	24
2200	2240	1600	0.28	61	5.5	37
2400	2450	1780	0.30	160	9.4	56
2500	2560	1860	0.30	240	12	68
2600	2660	1950	0.31	350	14	81
2700	2770	2030	0.32	500	17	96
2800	2880	2120	0.32	700	20	110
2900	2990	2200	0.33	950	24	130
3000	3090	2290	0.33	1260	27	150
3100	3200	2370	0.34	1650	31	180
3200	3310	2460	0.34	2100	35	200
3300	3420	2540	0.34	2700	39	230
3400	3530	2620	0.35	3400	43	260
3500	3650	2700	0.35	4200	46	300
3655 <sup>a</sup>	3820	....	0.35	5700	53	360

<sup>a</sup> Melting point of tungsten.

The spectral-energy-distribution curves of Fig. 3-7 cover the range of filament temperatures commonly encountered in commercial lamps. The wave length of maximum spectral emittance shifts from about  $1000\text{ m}\mu$  at  $2600^\circ\text{K}$  to  $800\text{ m}\mu$  at  $3300^\circ\text{K}$ . Also, with increasing temperature, the near ultraviolet increases much more rapidly than the infrared.

*Lamp Life.* The visible flux of an incandescent lamp decreases almost linearly with time until ultimately the filament burns out. The gradual decrease in output is due to evaporation of the filament, which results in decreased filament cross section, and to blackening of the bulb or envelope. The rate of evaporation increases rapidly with temperature, as do also the color temperature (whiteness of the light) and luminous efficiency. The lamp design is therefore always a compromise between efficiency and

life. Any factor that retards filament evaporation makes it possible to use a higher temperature and thus to secure higher radiation efficiency.

The evaporation of tungsten filaments is greatly retarded by filling the envelope with an inert gas and by coiling the filament. For the larger lamps an inert-gas mixture of about 0.8 atm of 80 to 90 per cent argon and 10 to 20 per cent nitrogen is used. Although the gas mixture increases thermal losses, the layer of stagnant gas around the filament

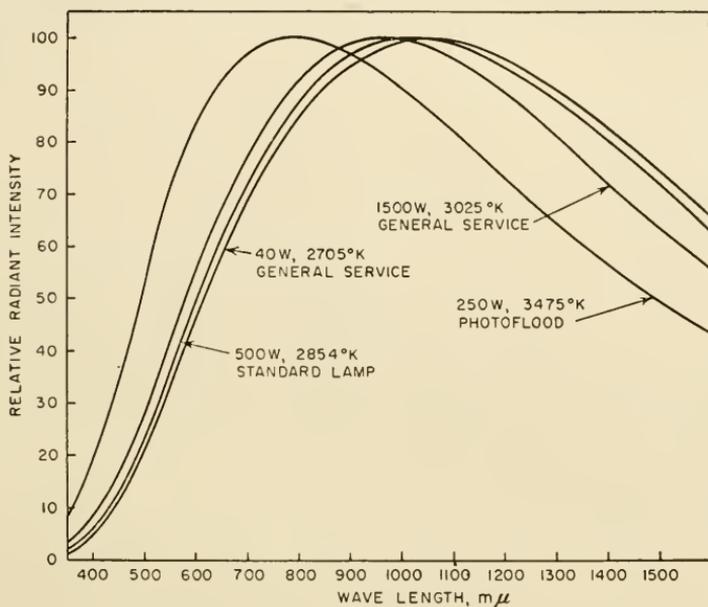


FIG. 3-7. Spectral emission of various types of tungsten-filament lamps. (Data from Forsythe and Adams, 1945.)

retards the rate of evaporation of the tungsten. The filament of a gas-filled lamp can be operated for the same useful life at a higher temperature (2800°–3000°K) than the vacuum lamp (2500°K), and the resulting increase in luminous efficiency more than compensates for the increased thermal losses.

The proportionate rate of evaporation from a fine wire is greater than that from a large wire because of the more favorable volume/surface ratio of the latter. Therefore, for the same life, low-voltage lamps can be operated at a higher temperature than high-voltage lamps. The optimum efficiency is at about 12 v for standard lamps since, at this voltage, large-diameter low-resistance filaments are required. Coiling also tends to retard evaporation, and the coiled-coil design used in low-wattage, high-voltage lamps, in which the wire is first coiled on a small mandrel and recoiled on a larger mandrel, involves an extension of this principle.

The ribbon-filament lamp is a source of very uniform intensity over the center portion of the ribbon. However, it cannot be maintained at

so high a temperature as the coiled-wire filaments for the same life. Since the ribbon must be thin to have sufficient resistance even for low voltages, holes quickly form at high temperatures. Therefore the ribbon filament is not usually employed where the highest intensity and color temperature are required.

*Electrical Characteristics.* The electrical characteristics of tungsten-filament lamps have been extensively covered in several reports (Bourne, 1948; Forsythe and Adams, 1936; Forsythe and Watson,<sup>1</sup> 1932, 1934; Jones and Langmuir, 1927a,b,c; Weitz, 1950). The voltage rating of an

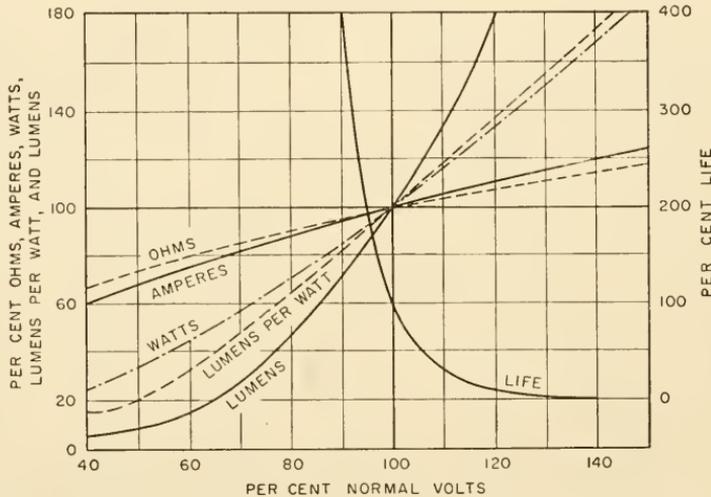


FIG. 3-8. Effect of voltage variation on the characteristics of tungsten-filament lamps. (From Weitz, 1950.)

incandescent lamp is selected to give certain radiation characteristics and lamp life, both of which change rapidly when the voltage is varied. For the average general-service type of lamp ( $T_e = 2800^\circ\text{--}3000^\circ\text{K}$ ) an increase of 1 per cent in voltage results in an increase of approximately 0.5 per cent in current, 1.5 per cent in wattage, 3.5 per cent in visible flux, and 2 per cent in luminous efficiency, but the life is decreased 13 per cent. If the voltage is increased 20 per cent, the luminous output is increased 85 per cent, but the life is reduced 90 per cent. These characteristics are presented graphically in Fig. 3-8. An increase in the temperature of a thermal radiator results in a shift of  $\lambda_m$  toward the shorter wave lengths. Consequently an increase in temperature causes the spectral energy to increase more rapidly in the shorter wave lengths than in the longer wave lengths. Using the equation given by Judd (1950) for a 500-w projection lamp operating at a color temperature of  $2800^\circ\text{K}$ ,

$$\% \frac{dV}{V} = \frac{200dT_c}{T_c - 620'} \quad (3-18)$$

it is possible to calculate the change in color temperature  $dT_c$  and from this the change in spectral emission of tungsten for small changes in voltage  $dV/V$ . A 1 per cent increase in voltage causes a  $12^\circ\text{K}$  increase in temperature, which results in the following increases in spectral emission: 1 per cent at  $4000\text{ m}\mu$ , 2 per cent at  $1000\text{ m}\mu$ , 2.5 per cent at  $800\text{ m}\mu$ , 3.5 per cent at  $600\text{ m}\mu$ , and 7 per cent at  $350\text{ m}\mu$ . It is thus evident that large gains in radiant-energy output can be obtained in the near ultraviolet by operating filaments at maximum voltage. In the infrared the increase in intensity is approximately proportional to the change in voltage.

The voltage stability required of a power source to maintain a specified stability in radiant flux at various wave lengths can be determined from these data. To keep the radiant-flux variation to within 1 per cent in the infrared, the voltage instability must be equal to or less than 1 per cent; in the visible the voltage instability must be less than 0.3 per cent; and in the ultraviolet, less than 0.1 per cent. This accounts in part for the instability frequently encountered in the ultraviolet when a spectrophotometer is operated with an incandescent source. Even a storage battery is difficult to maintain with a voltage instability of less than 0.1 per cent. An instrument that may be sufficiently stable in the infrared may be quite unstable in the ultraviolet.

Tungsten has a high temperature coefficient of resistivity, which also increases with temperature. The resistivity at room temperature, or  $300^\circ\text{K}$ , is  $5.64\text{ }\mu\text{ohm-cm}$  and at  $3000^\circ\text{K}$  is  $96.2\text{ }\mu\text{ohm-cm}$ , an increase of 17 times. As a result, there is a large inrush current at the instant of applying power to an incandescent lamp. In actual practice the inrush current is 8–12 times the normal operating current. With high-wattage lamps it is essential that the power and switching equipment be capable of handling these large currents. For example, a 1000-w 120-v lamp with a normal current of 8.3 amp has an inrush current of 65 amp, which can damage small switch contacts normally able to carry the rated current of the lamp.

*Application.* The most common applications of incandescent lamps in biological research are the general irradiation of biological material and the use with optical systems where it is essential to obtain the maximum energy through a relatively small aperture.

For general irradiation, bare lamps, either with clear or inside-frosted envelopes, may be used with reflectors. The type of reflector to use depends upon the general flux distribution required. This information is generally available from manufacturers of lighting equipment.

The internal-reflector lamp has greatly simplified the problem of general irradiation of large areas, since no external reflector is required. For use in greenhouses for supplementing solar irradiation, the internal-reflector lamp is much superior to other types, since it introduces a negligible shade

factor during the daylight period (Withrow and Withrow, 1947). In small compartments where plants are grown under high irradiances of total artificial radiant energy, several internal-reflector flood lamps allow better control of the irradiance than is possible where a single high-wattage lamp with a large reflector is used.

The projection lamp is designed for use with optical systems that require small sources of the highest and most uniform intensity (Bourne, 1948; Weitz, 1950). To achieve this, the filament is a short rod (Beutler and Metropolis, 1940) or is arranged in tight closely spaced coils and operated at the highest temperature consistent with a reasonable life. The coils are usually arranged parallel to one another and as close as possible without touching. When arranged in a single plane (monoplane filament), the brightness varies from a maximum at the coil to zero between the coils. If the parallel coils are arranged in two planes so that the coils of one plane are behind and between those of the other, the brightness becomes more uniform. Another modification is the "solid-source" design of Aldington (1945), in which the coils are arranged electrically in parallel on a low-voltage circuit. Since corresponding points on adjacent coils are at the same potential, the coils can be arranged so close together that they actually touch. The average intensity and brightness resulting from the biplane and "solid-source" filament constructions closely approach those of the filament wire.

The internal-reflector spot, or concentrating, lamp is one of the most efficient sources for the intense irradiation of small areas. A 300-w reflector spot lamp can produce irradiances of over 10,000 ft-c. Such high values are difficult to obtain with a 1000-w projection lamp and condenser system. The high efficiency of the concentrating reflector lamp is due to its relatively high effective-aperture ratio. The radiant energy from the filament is collected by the parabolic reflector through a very large angle. Unfortunately the optics of the molded bulb are not perfect enough for use in refined optical systems, but for the production of high-beam intensities with small, compact, and inexpensive equipment, the concentrating reflector lamp is unsurpassed.

The principal limitation on the use of the incandescent lamp for the irradiation of biological materials with visible irradiances of over a few hundred foot-candles is the high proportion of the total energy which is radiated. It is evident that all the thermal sources radiate most of their energy in the infrared (see Table 3-13). For the general-service incandescent lamp, 83-94 per cent of the input electrical energy is radiated. Of the total radiated energy, only about 10 per cent is in the visible.

If a water filter is used to absorb the infrared, the resulting transmitted energy has a higher luminous efficiency (Gordon, 1930). For a 1-cm layer of water in a glass cell with parallel faces, the visible energy is reduced about 10 per cent, largely by reflection from the air-glass and water-glass

interfaces, and the total energy is reduced 77 per cent. The transmitted energy is increased in luminous efficiency by a factor of nearly 4. A 10-cm water filter increases the luminous efficiency nearly six times.

#### MISCELLANEOUS INFRARED SOURCES

Any thermal source of radiant energy is an efficient source of infrared by virtue of the very high proportion of energy emitted in the infrared. The wave length of maximum energy  $\lambda_m$  for the short-lived photoflood lamp at 3360°K is about 0.8  $\mu$ ; for the general-service lamp at 2900°–3100°K,  $\lambda_m$  is about 0.9  $\mu$ , and the life is 1000 hr. At 2200°–2500°K the

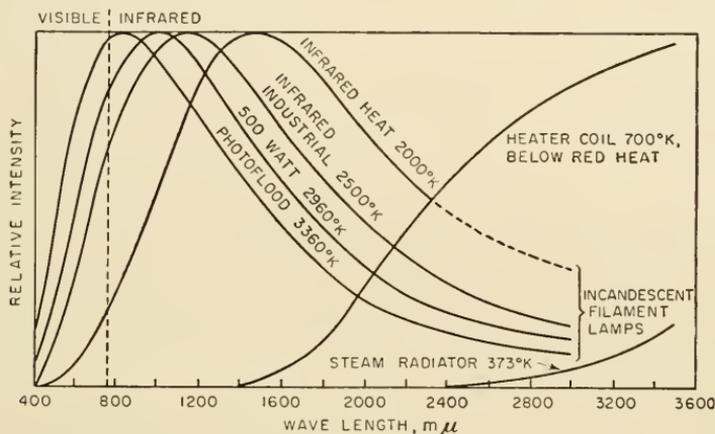


FIG. 3-9. Relative spectral energy distribution of infrared sources. (From *IES Handbook*, 1952.)

$\lambda_m$  is extended to 1.0 or 1.2  $\mu$ , and the life to several thousand hours. This is the operating condition for the industrial infrared lamp used for drying materials and baking industrial finishes. Low-temperature emitters, such as the carbide or Globar emitter, operating at 1000°–1400°K, are used extensively in infrared spectrophotometry (Taylor *et al.*, 1951) and as industrial low-temperature sources. The spectral energy distributions of various infrared sources are given in Fig. 3-9.

Plant and animal tissues are chiefly water and therefore transmit little infrared energy beyond 1.5  $\mu$ . Practically all the energy of the longer infrared wave lengths is dissipated in the tissue surfaces. Mammalian animal tissues and chlorophyllous plant tissues also absorb strongly in the visible, owing principally to the presence of porphyrins and other pigments. The only region of high transmission is in the near infrared from about 0.7 to 1.5  $\mu$ . Therefore the general-service incandescent lamp with its maximum emission at about 0.9  $\mu$  is the most efficient source for obtaining maximum penetration of energy and the heating of tissues to an appreciable depth.

## GASEOUS DISCHARGE LAMPS

## GENERAL CHARACTERISTICS

The gaseous discharge lamps fall into three general classes, depending on the operating pressure of the conducting and radiating gas (Bourne, 1948; Forsythe *et al.*, 1941; Koller, 1952). The low-pressure lamps have a gas or vapor pressure of between 0.001 and 10 mm Hg; the medium-pressure lamps, from 0.5 to 30 atm; and the high-pressure lamps, from 30 to several hundred atmospheres. The low-pressure discharge is characterized by a diffuse luminous glow of low intensity; the spectrum consists of sharp lines with little or no continuous energy between the lines, and at the lowest pressures most of the energy may be in the resonance lines of the element. The medium- and high-pressure discharge lamps usually have a sharply defined arc stream of high intensity. With increasing pressure the lines become complex and broadened, and a continuum appears between the lines owing to the high temperature of the ionized gas, and at extreme pressure and current density the line spectrum may be masked completely by the continuum.

The medium-pressure arc may be operated as an open arc in air at 1 atm or as an enclosed arc. The open carbon arc has almost unlimited power input. However, the air gases cause the rapid erosion of the electrodes, and complicated electrode feed mechanisms are required for stable operation.

*Cathodes.* Most gaseous discharge lamps can be designed to operate on either direct or alternating current. On direct current one electrode serves as the cathode or negative terminal, and the other as the anode or positive terminal. The cathode supplies the electrons, which are accelerated by the potential gradient. On alternating current each electrode is a cathode for half the cycle and an anode for the other half. Therefore the electrodes are usually asymmetrical in d-c lamps, with only one electrode as the electron emitter, but in a-c lamps both electrodes are emitters.

The discharge may be excited between either cold cathodes of large area or hot cathodes of relatively small area. The cold cathode is less efficient but relatively indestructible. It is used principally in lamps that are operated intermittently, as in low-pressure high-voltage sign lighting and flash tubes. The cold cathode is less efficient than the hot-cathode emitter because of the large losses resulting from the high cathode potential drop.

The emission of electrons from a cathode surface is dependent upon the work function of the surface and the temperature. To be an efficient emitter, pure tungsten must operate close to its melting point, whereas the addition of a small amount of thorium greatly increases the electron emission and makes possible a lower operating temperature. Oxides of the rare earths, especially those of barium and strontium, are the most

efficient emitters and may be operated at relatively low temperatures. The oxide-coated cathode is used chiefly in the low- to medium-pressure discharges, and the more rugged thoriated-tungsten and pure-tungsten cathodes are employed in the high-pressure arcs.

The low-pressure discharge tubes, such as the fluorescent lamps, are commercially available with both cold and hot cathodes; the latter are manufactured as preheat and instant-start types. In the preheat cathode a current is passed through a filament for a sufficient time to bring it to an efficient emitting temperature before the discharge is initiated. This

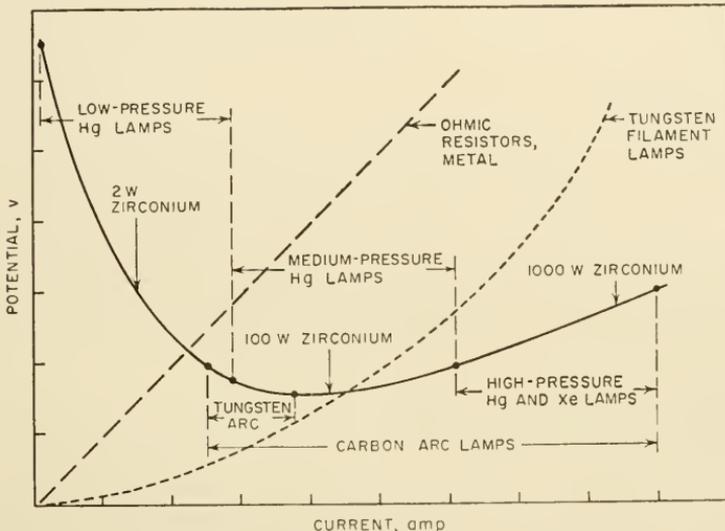


FIG. 3-10. The volt-ampere characteristics of arcs and ohmic resistors.

is usually accomplished by some type of automatic switch or starter which shorts the high voltage of the ballast through the cathodes for a few seconds. After the cathodes are heated, the switch opens, producing a high-voltage impulse which initiates the discharge. The instant-start lamp is powered by a ballast supplying a voltage sufficient to start the discharge with cold cathodes, which then quickly attain the normal emitting temperature. The instant-start lamp requires no complex starting mechanism, and there is no flickering after the lamp is switched on.

*Electrical Characteristics.* The electrical discharge sources have a negative electrical resistance characteristic. Ohmic resistors, such as those of metal, have a positive resistance characteristic; i.e., the current is proportional to the voltage, according to Ohm's law, and the plot of current as a function of voltage has a positive slope (Fig. 3-10). For a negative resistance the current decreases with increasing voltage, and the characteristic curve has a negative slope. When an ionic conductor such as a discharge lamp, with a negative resistance characteristic, is applied to a

constant-voltage source, the current increases rapidly until failure occurs in the power supply. Therefore all arcs and other forms of gaseous discharge sources require a stabilizing impedance to neutralize the negative resistance characteristic of the discharge and to give the over-all system a positive impedance. Such an impedance is known as a "ballast." Discharge lamps operating on direct current require resistive ballasts, but on alternating current they are usually stabilized with inductive ballasts. Capacitive ballasts may be used only at frequencies above about 300 cps (Campbell, 1948; Campbell and Bedford, 1947). At very high current densities the resistance characteristics of the arc may become zero or even positive. However, even when operating in the positive-resistance range of the characteristic curve, the arc requires a limiting impedance for stable operation.

If the discharge requires a voltage for starting which is above that of the line voltage, the current-limiting inductance and the step-up transformer are usually incorporated into one unit as a high-reactance transformer. As more current is drawn from the secondary, the secondary voltage of such a reactive transformer decreases nearly proportionately.

*Life.* The life of enclosed gaseous discharge lamps is determined by the rate of blackening of the envelope and deterioration of the cathode. The blackening of the envelope is largely due to the sputtering and evaporation of material from the electrodes and in some cases to chemical decomposition resulting from traces of water vapor or oxygen in the lamp. Deterioration of the oxide-coated cathodes results principally from evaporation and sputtering of active material from the surface. The most rapid rate of deterioration of oxide-coated cathodes occurs at the instant of starting, when the potential gradient is high. Therefore the life of such lamps is markedly influenced by the frequency of starting.

#### TUNGSTEN ARC

In an attempt to attain round small sources of high brightness, several forms of the tungsten arc have been developed under such names as the Pointolite, photomicrographic lamp, and the S-1 sunlamp (Bourne, 1948; Illuminating Engineering Society, 1952; Koller, 1952; Taylor, 1931; Weitz, 1950). These sources consist of tungsten electrodes, one or both spherical or cup-shaped, which are brought to incandescence by ion bombardment. The discharge is maintained in an atmosphere of argon or argon and mercury. Although these lamps are operated as gaseous discharge lamps, most of the radiant energy comes from the incandescent electrodes, and the spectrum is that of incandescent tungsten upon which is superposed a spectrum of the ionizing constituents. Since it is not necessary to maintain the complex mechanical shape of a filament, a somewhat higher temperature can be maintained than with filament lamps. Whereas in the filament projection lamp the brightness is seldom

higher than  $25 \text{ c mm}^{-2}$ , the G.E. photomicrographic lamp operates with a brightness up to  $48 \text{ c mm}^{-2}$ , which implies that the tungsten electrode is within  $100^\circ\text{C}$  of its melting point. In such a lamp, life is determined primarily by the blackening of the bulb. The photomicrographic lamps are used principally with optical systems. The S-1 lamp (Taylor, 1931) has sufficient mercury to produce considerable ultraviolet from the mercury-arc stream and is used chiefly for the production of therapeutic ultraviolet.

#### CARBON ARC

The carbon arc is a medium-pressure arc operating in air at a pressure of 1 atm between electrodes of carbon compounded with various other materials (Bourne, 1948; Finkelburg, 1949, 1950; Forsythe, 1940; Illuminating Engineering Society, 1952; National Carbon Company, 1944, 1948). It was the first electric-light source invented and is still the most intense source available for continuous operation. Modern lamps with well-designed feed mechanisms and magnetic-field stabilization and using cored carbons of uniform composition have a high degree of stability and are quiet. The brightness of a high-intensity arc operating under favorable conditions will fluctuate less than 5 per cent over considerable periods of time. The low-intensity arc (Rupert, 1952) can have a fluctuation of less than 2 per cent.

The spectrum of the carbon arc consists of thermal radiation, of  $3800^\circ\text{K}$  color temperature, from incandescent carbon in the positive-electrode crater upon which are superposed the line and band spectra of core materials and substances formed by the reaction of air with carbon. Pure-carbon electrodes produce an arc with a strong series of "cyanogen" bands in the region between 380 and 400  $\text{m}\mu$  and another wide band at 250  $\text{m}\mu$  (Bowditch and Downes, 1938; Coblenz *et al.*, 1926; Greider and Downes, 1932; National Carbon Company, 1944) (Fig. 3-11).

In d-c arcs most of the incandescence comes from the crater formed in the end of the positive electrode. The negative electrode is smaller in diameter and burns at a much slower rate, and the tip becomes pencil-shaped. Various mechanisms have been devised for controlling the rate of feed of the individual carbons so as to keep the arc length constant and the crater fixed in position.

All modern carbon arcs employ cored-carbon electrodes which consist of a tube of hard carbon filled with soft powdered carbon mixed with various inorganic salts. The softer core tends to stabilize the arc stream in the center of the electrode and provides a convenient means for adding other compounds. Potassium salts are added as arc-supporting materials which contribute little to the radiation but, because they ionize readily at high temperatures, stabilize the arc. Salts of the rare earths, particularly those of the cerium group, produce an incandescent white

flame that greatly increases the brightness of the arc. Salts of other metals are used to accentuate various portions of the spectrum.

In order to reduce the voltage drop along the carbon and consequent overheating at high current densities, the carbons are often copper-coated to increase their electrical conductance. As the carbon burns, the copper burns off at the same rate. The positive rotating carbon, in many high-intensity lamps, is not copper-coated, but the carbon is clamped between

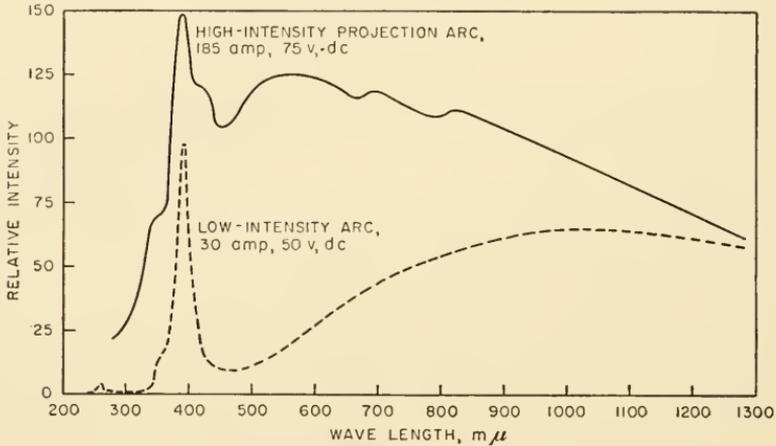


FIG. 3-11. Relative spectral energy distribution of projection types of carbon arcs. (Data from Greider and Downes, 1932.)

metal jaws close to the tip so that the current path is short. In these lamps only the negative electrode is copper-coated.

The rate of burning of the electrodes is largely a function of current density and air movement. The rate of positive carbon feed varies from 5 to 60 cm hr<sup>-1</sup> in standard equipment (Illuminating Engineering Society, 1952; National Carbon Company, 1948). Standard carbon diameters range 5-16 mm, and lengths, 20-55 cm.

The a-c arc employs symmetrical cored carbons in which both electrodes radiate equally. The a-c arc is less intense than the d-c arc of equivalent power because each electrode is positive only half the time (Joy and Geib, 1934).

The arc is usually initiated by bringing the carbons together and then separating. At the point of contact the carbon becomes incandescent and is capable of emitting sufficient electrons to start the arc at the instant of separation. The arc may also be started by a third high-voltage electrode. All d-c arcs at low to medium power have a negative resistance characteristic and require a stabilizing resistor. At very high current densities the volt-ampere characteristic becomes positive, and less ballast is needed in this region. In general, the most stable operation is obtained when the supply voltage is 20-30 per cent above the arc voltage. The

difference between supply and arc voltages is consumed in the stabilizing resistor.

There are three classes of carbon arcs, based upon the nature of the coring material and the current density. These are the low-intensity, high-intensity, and flame arcs.

*Low-intensity Arc.* Solid or neutral-cored carbons containing potassium salts for supporting the arc are used in the low-intensity arc. The current density is 8–30 amp  $\text{cm}^{-2}$ . Most of the energy comes from the crater formed in the positive carbon which has an actual temperature of about 3900°K, the temperature of sublimation of carbon. Increasing the current density does not appreciably increase the core temperature or the brightness of the low-intensity arc, since carbon sublimates without melting; increasing the current merely increases the size of the crater and the rate of burning. The crater brightness is relatively constant at 175–180 c  $\text{mm}^{-2}$  and is nearly independent of current density.

The low-intensity arc is frequently semienclosed for industrial operation. The arc quickly uses up the oxygen within the enclosure, and the atmosphere becomes chiefly carbon dioxide and nitrogen at atmospheric pressure. This greatly accentuates the cyanogen bands in the blue and near ultraviolet and retards the rate of burning of the electrodes but decreases the luminous efficiency.

These arcs are operated in current ranges of 10–30 amp. Increasing the arc voltage by lengthening the arc gap accentuates the ultraviolet cyanogen bands. Arcs of this type are useful for blueprinting and other applications requiring high blue and near-ultraviolet flux. The low-intensity arc is probably the most uniform and reproducible of the three classes and has been used as a source for infrared spectroscopy (Rupert, 1952).

*High-intensity Arc.* In the high-intensity arc the core contains flame-producing materials, principally cerium salts, in addition to the arc-supporting compounds (Bowditch and Downes, 1938; Finkelburg, 1949; Gretener, 1950; Jones and Bowditch, 1949; Zavesky *et al.*, 1945). The current densities range from 60 to 200 amp  $\text{cm}^{-2}$ . Most of the radiant energy (70–90 per cent) comes from a highly incandescent vapor cloud of cerium salts in the crater. The incandescence of the vapor cloud is due to its extremely high temperature of 7000°–8000°K (Finkelburg, 1949, 1950; Forsythe, 1940). Unlike the low-intensity arc, the brightness of the crater increases with current density; values up to 2500 c  $\text{mm}^{-2}$  have been produced experimentally (Finkelburg, 1949; Gretener, 1950); commercial lamps produce values of 350–1200 c  $\text{mm}^{-2}$ .

The current requirement of standard high-intensity-projection-arc equipment is from 150 to 225 amp (Illuminating Engineering Society, 1952; National Carbon Company, 1948). The arc voltage is relatively constant and varies from 70 to 80 v, depending upon the current. The

high-intensity arc is used extensively with optical systems such as motion-picture projectors, theatrical spotlights, and searchlights and for experimental purposes requiring the highest radiation intensities. The two most common types are the reflector lamps for medium power, with automatic-feed and nonrotating carbon electrodes mounted on the reflector axis, and high-power condenser-lens equipment with rotating positive electrodes, with the negative carbon displaced about  $60^\circ$  from the axis. The condenser-lens type of equipment collects the flux without introducing shadows in the beam, as reflecting optical systems do. The most stable operation is obtained with the automatic-feed rotating positive carbon lamp. This source is probably the most satisfactory for optical systems requiring intense irradiation of small areas with uniform flux, as in the irradiation of a monochromator slit.

*Flame Arc.* The cores of flame-arc carbons contain large quantities of rare-earth salts and other materials to accentuate certain regions of the spectrum. In the white-flame arc the salts are principally cerium. Calcium produces a yellow flame; strontium, a reddish flame; and the poly-metallic cores containing iron, nickel, cobalt, and aluminum produce flames especially rich in ultraviolet energy in the region of  $300\text{ m}\mu$  (Greider and Downes, 1932; National Carbon Company, 1944).

Owing to the activity of the core material, the arc produces a large flame which is responsible for most of the radiant energy and luminosity. The emission spectrum depends chiefly on the composition of the flame-producing materials. The flame arc is the most flexible of all carbon arcs in regard to spectral characteristics.

Since most of the luminosity is in the flame, the intensity of the flame arc is much less than that of the other two types (see Table 3-14). Low current densities are used, and alternating current is a common source of power, since high intensity is not the principal objective. These lamps are used principally for general irradiation in photochemical industrial processing and as therapeutic sources for artificial sunlight.

#### ZIRCONIUM OR CONCENTRATED ARC

This source consists of a d-c arc between a cathode of a metallic film of zirconium or zirconium oxide and an anode of refractory metal (Buckingham and Deibert, 1946a,b). The zirconium oxide is packed into a tube of refractory metal such as tantalum, and in the enclosed arc the discharge takes place to a refractory metal ring, as shown in Fig. 3-12. Zirconium oxide has a melting point in the vicinity of  $3000^\circ\text{K}$  and is maintained in the molten state by the discharge. Some of the oxide is reduced to zirconium metal as a thin film on the molten surface. The enclosed lamps are operated in an atmosphere of argon, and the arc is initiated by a high-voltage discharge and is sustained as a low-voltage arc.

Zirconium enclosed arcs are manufactured in power ratings of 2-1000 w.

The 2-w lamp has a source diameter of 0.085 mm (0.003 in.), a maximum brightness at the center of  $96 \text{ c mm}^{-2}$ , and an average brightness of  $56 \text{ c mm}^{-2}$ . As the wattage is increased, the dimensions of the source are likewise increased, but the maximum brightness decreases. The 100-w lamp has a maximum brightness of  $52 \text{ c mm}^{-2}$  and a source diameter of 1.5 mm. The zirconium arc has a brightness considerably higher than that of the tungsten-filament lamp but is not so stable a source. The arc has a tendency to wander over the cathode surface and to give small variations in image brightness. The spectral energy distribution is

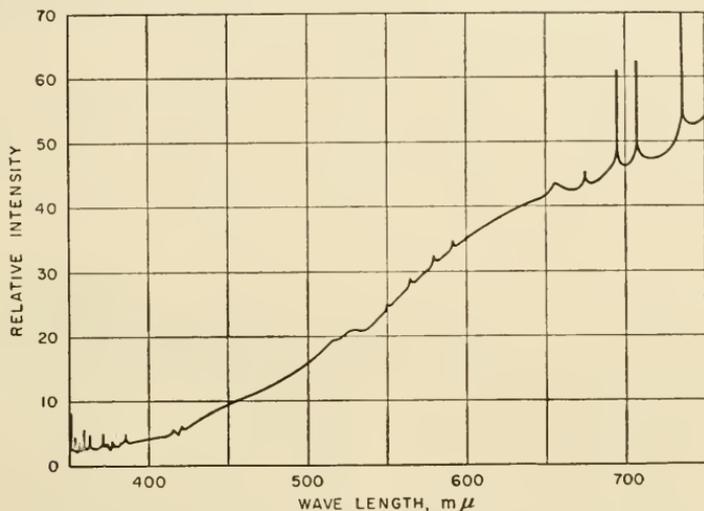


FIG. 3-12. Spectral emission of an a-c zirconium concentrated-arc lamp. (From Buckingham and Deibert, 1946a.)

that of a thermal radiator upon which are superposed the weak line spectra of zirconium and argon. The color temperature is about  $3600^\circ\text{K}$ . The open arcs are operated in air and consist of a mixture of zirconium oxide and nickel powder in a nickel tube. These lamps are available in powers ranging from 300 to 1000 or more watts.

The radiation of the zirconium arc may be modulated with an alternating current superposed on the direct current at all frequencies throughout the audio-frequency range (Buckingham and Deibert, 1947). The modulated radiation can be detected with photocells and a-c amplifiers. This makes it possible to use them as modulated sources in a-c radiometry.

The 2-w enclosed lamp is a close approximation of a point source and is ideal for shadow photography. It can be used without any optical components to produce very sharp, highly magnified shadow images.

#### HYDROGEN ARC

An arc discharge in hydrogen produces a continuous spectrum that extends from the vacuum ultraviolet into the visible. It is an excellent

source of continuous radiation in the ultraviolet; the visible emission is relatively weak. For this reason the hydrogen arc has been used extensively for ultraviolet spectrophotometry. The hydrogen arc has been produced in various forms from low-pressure cold-cathode lamps operating at high voltages to the relatively high-pressure low-voltage hot-cathode type of arc (Finkelstein, 1950; Smith and Fowler, 1936). It is also available commercially as low-wattage hot-cathode sources for spectrophotometry. The arc described by Smith and Fowler has a hot cathode and operates on direct current at a pressure of 0.2 mm hydrogen and currents ranging from 0.5 to 20 amp. A voltage of 220 de is adequate to supply the ballast and arc requirements.

Even at relatively high currents the hydrogen arc is a rather diffuse low-intensity source. Therefore most of the lamps are of the end-on type, in which the energy comes from a considerable depth of discharge. The high-power lamps require water cooling even when quartz envelopes are used.

#### XENON ARC

The high-pressure compact xenon or Schulz arc (Aldington, 1949; Anderson, 1951; Schulz, 1947) has a spectral energy distribution very rich in blue and ultraviolet and somewhat similar to that of the high-intensity carbon arc (Fig. 3-13). The arcs described by Anderson contain xenon at a pressure of 20–44 atm in a very thick spherical quartz envelope and have tungsten electrodes separated by 3.5–6.5 mm. Lamps of 150–1000 w are available which, though not having the brightness of the mercury arc (Table 3-14), have a much more uniform spectrum containing a large number of closely spaced lines and a continuum. Brightness values of nearly  $200 \text{ c mm}^{-2}$  have been obtained, as compared with

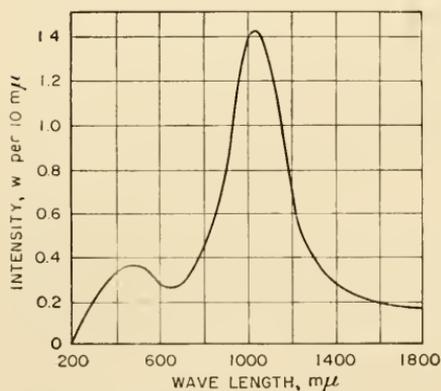


FIG. 3-13. Spectral emission of a 150-w high-pressure xenon a-c arc at 23 atm and 3-mm arc length. (From Anderson, 1951.)

$350 \text{ c mm}^{-2}$  for the mercury high-pressure short arc. The electrodes become incandescent and contribute an appreciable amount of infrared and visible energy. The xenon compact arc is an excellent source for use with optical systems because of its high brightness and small dimensions. The xenon arc has considerably higher ultraviolet intensity from 250–350  $m\mu$  than does a low-intensity carbon arc (Baum and Dunkelmann, 1950), and it is more stable than the carbon arc.

## MERCURY ARC

In the mercury-arc discharge lamp the distribution of energy into the various excited states of the mercury atom is in part determined by the vapor pressure. These lamps may be divided into three general classes depending on the operating vapor pressure. The low-pressure lamps are low-intensity sources in which most of the energy is in the ultraviolet and the spectrum consists of relatively few sharp lines (Table 3-11).

TABLE 3-11. SPECTRAL ENERGY DISTRIBUTION OF MERCURY-ARC LAMPS

Wave length, $m\mu$		Lamp wattage and designation <sup>a</sup>			
		100 H-4	250 H-5	400 EH-1	250 UA-2
Spectral band	Principal lines	Average watts radiated per 10 $m\mu$ within wave band indicated			
220-230	—				0.4
230-240	—				1.6
240-250	—				1.7
250-260	254				4.5
260-270	265				2.7
270-280	—				0.9
280-290	280				1.0
290-300	297	0.01			1.5
300-310	302	0.07			2.6
310-320	313	0.64		0.07	5.3
320-330	—	0.22	0.02	0.06	0.3
330-340	—	0.39	0.10	0.23	0.7
340-350	—	0.18	0.10	0.18	0.2
350-360	—	0.13	0.14	0.22	0.1
360-370	365	3.3	6.0	8.8	7.0
370-380	—	0.14	0.27	0.50	0.2
380-400	—	0.11	0.18	0.25	0.1
400-410	405	1.30	3.5	6.8	2.5
410-430	—	0.13	0.31	0.6	0.2
430-440	436	2.4	6.9	12.7	4.0
440-540	—	0.06	0.2	0.3	0.1
540-550	546	2.9	8.6	15	4.6
550-570	—	0.06	0.18	0.6	0.1
570-580	578	2.3	8.8	16	5.0
580-760	—	0.02	0.13	0.3	0.06

<sup>a</sup> The H lamps have a high-silica glass tube inside a glass jacket; the UA lamp consists of a single tube of quartz.

Medium- to high-pressure lamps operate at pressures from a half to several hundred atmospheres. They are high-intensity sources whose spectral energy distribution is shifted toward the visible; the lines are broadened, and a continuous spectrum is superposed on the line spectrum. The various types of mercury arcs have been discussed in detail by several authors (Barnes and Forsythe, 1936; Bourne, 1948; Forsythe and Adams, 1948; Forsythe *et al.*, 1942; Illuminating Engineering Society, 1952; Koller, 1952; Weitz, 1950).

*Low-pressure Discharge.* At very low vapor pressure the mean free path between the atoms is sufficiently large so that there is a high probability that most of the energy will be radiated in the resonance line at  $253.7\text{ m}\mu$ , which represents the transition from the lowest excited state to the ground state in the mercury atom. The efficiency of production of the resonance radiation is highest when the pressure is between 0.008 and 0.10 mm Hg, which corresponds to an envelope temperature of about  $45^{\circ}\text{C}$ . Under optimum conditions as much as 60 per cent of the electrical input energy may appear as resonance radiation at  $253.7\text{ m}\mu$ , an amazingly high efficiency for conversion of electrical to radiant energy. Very little of the radiated energy (less than 3 per cent) appears in the other mercury lines of the near ultraviolet and visible. As the pressure is increased to several millimeters, a larger proportion of the energy appears in the near-ultraviolet and visible lines, but the lines are still sharp, and there is almost no continuum (Forsythe and Adams, 1948). As is common with all low-pressure low-current-density discharges, the intensity is low and the energy is diffusely distributed. Consequently, high power involves large distributed sources.

*Medium-pressure Arc.* As the vapor pressure is increased by a higher operating temperature, the discharge becomes increasingly concentrated, the current density increases, and an increasing proportion of the radiated energy appears in the visible and near ultraviolet as a continuum. Many of the medium-pressure lamps operating at 0.5–10 atm have a small arc tube surrounded by a second envelope. The space between the two envelopes is evacuated so as to reduce heat losses from the arc tube. A limited amount of mercury is introduced which is just sufficient to maintain the proper operating pressure when completely vaporized.

*High-pressure Capillary Arc.* The highest intensities are produced by the high-pressure capillary arc in which the pressure is from 30 to several hundred atmospheres and the temperature so high that even with fused quartz the tube must be cooled by an air blast or by flowing water (Bourne, 1948; Weitz, 1950). In Fig. 3-14 are given the spectral-energy-distribution curves of a series of capillary arcs operating at pressures of 31–285 atm. It will be noted that at the highest pressure the continuum between the lines has become the predominant feature of the arc, whereas

at the lowest pressure the continuum is weak and most of the energy is in the lines.

The capillary arc is limited to about 1 kw for a capillary of 2 mm. Since the energy is concentrated within the confines of the capillary, it makes an excellent source for the irradiation of a monochromator slit.

*Short, or Concentrated, Arc.* In order to obtain a source of more nearly spherical dimensions and one not limited by the melting point of quartz,

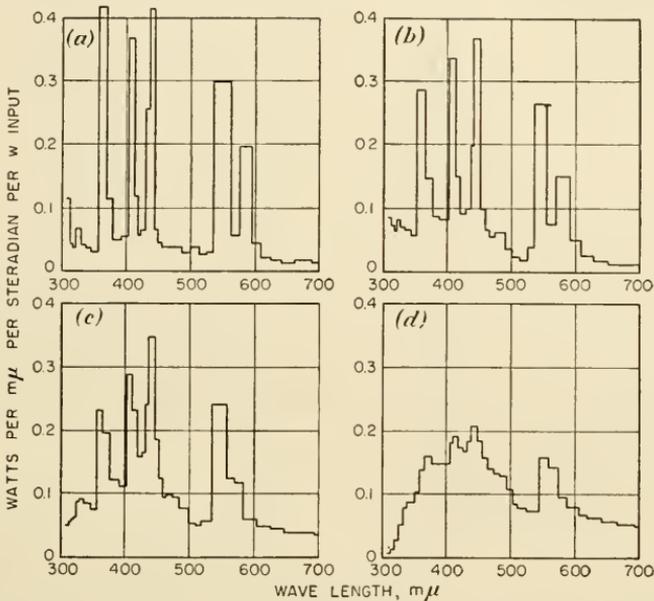


FIG. 3-14. Effect of mercury vapor pressure on the spectral energy distribution of high-pressure capillary mercury arcs. Curve *a*, 54 atm; curve *b*, 102 atm; curve *c*, 197 atm; curve *d*, 319 atm. (From Forsythe, Adams, and Barnes, 1942.)

the so-called “short-” or “concentrated-arc” lamp has been developed (Anderson, 1951; Freeman, 1950). Massive tungsten electrodes, within a large thick-walled spherical quartz envelope containing a small amount of mercury, confine the arc to a short stream about 10 mm in length and 5–10 mm in diameter. These lamps may be started automatically with a high-voltage pulse circuit. Since the quartz envelope is some distance from the arc stream, it is not exposed to excessive temperatures. This lamp is made in sizes up to 10 kw. The spectral characteristics of the short-arc lamp are very similar to those of the high-pressure capillary arc.

*Operation.* Since liquid mercury at room temperature has too low a vapor pressure to support a discharge at reasonable exciting potentials, argon gas at several millimeters pressure is usually present. The discharge is initiated in argon, and then, as the temperature rises and the

mercury pressure increases, ionized mercury takes over the conduction of the current. Modern lamps start automatically without mechanical tilting. The medium-high-pressure mercury arcs do not restart immediately after being extinguished; the lamp must cool sufficiently so that the vapor pressure is low enough for restarting. This requires up to 10 min, depending on the ambient temperature and the type of lamp. Restarting time for the capillary arc is only a few seconds owing to the rapid dissipation of heat by the air blast or cooling water.

*Application.* Because of the very high efficiency of production of ultraviolet energy at  $253.7\text{ m}\mu$ , the low-pressure mercury arc is used as a source of germicidal energy and for the excitation of phosphors in the fluorescent lamp. The medium-pressure arcs are especially useful as intense sources of monochromatic energy when used with filters. At medium pressure the background of continuous radiation is not excessive, and nearly all the energy is in the lines. They have been used to supplement the radiation of the incandescent lamp, which is deficient in the blue. They have not proved very satisfactory for the growing of plants, principally because all mercury arcs are relatively deficient in red energy. Comparative tests have shown that the mercury arc is considerably less efficient than the incandescent or fluorescent lamp for plant growth (Withrow and Withrow, 1947).

The high-pressure arcs are especially useful as optical sources of high intensity. The capillary arc is very efficient with a monochromator because of its linear dimensions and very high intensity. The thermal-radiation continua of the capillary- and the short-arc lamps are sufficiently great so that monochromatic flux of high intensity can be isolated from any region of the visible and ultraviolet.

#### AMALGAM ARC

Although the mercury arc is a source of high brightness and luminous efficiency, it is limited for many applications because of its deficiency in the red. Operation at high pressures partially supplements the red deficiency by a continuum, but the red flux is still weak in proportion to the shorter wave lengths. Efforts to supplement the red by the use of mercury amalgams of metals with strong red emission lines have proved partially successful. Cadmium with strong visible lines at 468, 480, 509, and  $644\text{ m}\mu$  and zinc with lines at 472 and  $636\text{ m}\mu$  amalgamate readily with mercury, and their emission spectra appear along with the spectrum of mercury.

A capillary arc at 100 atm with an amalgam of 13 per cent cadmium and 17 per cent zinc produces a source that closely approximates sunlight in color (Elenbaas and Riemens, 1950; Bourne, 1948). The principal limitation of such sources is relatively low power efficiency. This is of little consequence in experimental work, and such sources deserve more attention for photochemical investigations than they have received.

Cadmium, zinc, and thallium may be operated as pure metals in quartz tubes, with an outer insulating envelope very similar in design to the medium-pressure mercury arcs. The metal is limited in amount so that,

TABLE 3-12. WAVE LENGTH, IN MILLIMICRONS, OF PRINCIPAL SPECTRAL LINES OF THE METALLIC ARCS AND HELIUM DISCHARGE

Cadmium	Mercury	Thallium	Helium
228.8	184.9	270.9	273.3
326.1	194.2	276.8	318.8
340.4	253.6	291.8	320.3
346.6	313.2	323.0	388.9
361.0	365.0	351.9	468.6
467.8	366.3	352.9	587.6
480.0	404.7	535.0	
508.6	435.8	655.0	
643.8	546.1		
1039.5	577.0	Zinc	
	579.1	280.0	
Cesium	1014.0	303.6	
455.5		328.2	
459.3	Sodium	330.3	
794.4	330.2	334.5	
807.9	568.8	468.0	
852.1	589.0	472.2	
894.4	589.6	481.0	
917.2	819.5	636.2	
	1138.2	1105.4	
	1140.4		

upon freezing, the volume is not sufficient to crack the quartz envelope. The wave lengths of the principal lines are given in Table 3-12.

#### ALKALI-METAL ARCS

The resonance lines of sodium are in the sodium doublet at 589.0 and 589.6  $m\mu$  and very close to the peak of sensitivity of the light-adapted eye. These lines are excited most efficiently under low pressure, where the mean free path of the sodium atoms is large. It is therefore impossible to obtain efficient sodium lamps of high intensity or brightness. However, the luminous efficiency of the sodium lamp is high, being approximately 50 lumens  $w^{-1}$  in commercial lamps (Buttolph, 1935). Since practically all the energy is in the sodium doublet, it is very useful as a source that is sufficiently monochromatic for many purposes.

The lamp consists of an inner bulb of special glass resistant to metallic sodium and an outer double-walled evacuated flask that thermally insulates the inner tube. Since metallic sodium is a solid at room temperature and the power input to the lamp is relatively small compared with its volume, it takes about 30 min for the lamp to come to optimum temperature and maximum brightness. The sodium lamps manufactured in the United States are available in 180- and 28-w sizes. The 28-w lamp

is used principally as a laboratory source of monochromatic energy. The lamp contains both a filamentous cathode and an anode at each end. Since neon is present for initiating the discharge, the radiation is characteristic of that of neon during the first few minutes of operation, after which sodium atoms take over the discharge. The characteristic line spectra of cesium, potassium, and rubidium may be obtained with lamps containing these elements separately (Beese, 1946).

#### MISCELLANEOUS DISCHARGE LAMPS

There are a number of discharge lamps of low power designed for specialized applications. When the lamps are of the high-voltage low-pressure type, they are known as "Geisler tubes." The so-called "glow lamps" are of the low-voltage low-pressure type. The radiation of the glow lamps can be modulated readily with alternating current. They are used as voltage-stabilizing elements in electrical circuits, since the voltage drop across them tends to be constant and characteristic of the ionization potential of the gas.

The helium tube is especially useful for the calibration of spectrophotometers, since there is only one strong line in the visible at  $587.6\text{ m}\mu$  (Table 3-12). It is an excellent monochromatic source for interferometry and other optical applications. The argon lamp emits radiation rich in ultraviolet, with relatively little visible, and consequently has been used without filtering as an ultraviolet source for exciting fluorescence. The neon lamp is high in red-orange energy and is available both as a low-pressure discharge in the conventional low-wattage glow lamp and as a moderately high-pressure discharge with oxide-coated cathodes. Lamps of this latter type have been manufactured in sizes of several hundred watts.

#### FLUORESCENT LAMPS

Ultraviolet resonance radiant energy at  $253.7\text{ m}\mu$ , produced by a low-pressure mercury discharge, is absorbed by a phosphor coating on the inner side of the fluorescent lamp tube and reemitted at longer wave lengths. The thin layer of phosphor crystals serves as an efficient radiation transformer, absorbing quanta of one frequency and reemitting quanta of a lower frequency in accordance with Stokes' law. A comprehensive treatment of the fluorescent lamp is presented by Forsythe and Adams (1948).

#### PHOSPHORS

The ideal phosphor coating absorbs all the  $253.7\text{-m}\mu$  energy and reemits that energy with a quantum efficiency of 1. Actual phosphors very nearly attain this ideal with quantum efficiencies of 0.76–0.90. Even with a quantum efficiency of 1, there is a large loss in radiant energy. As an example, the conversion of a high-energy ultraviolet quantum at

253.7  $m\mu$  to a low-energy quantum of visible at 507.4  $m\mu$  entails a 50 per cent energy loss.

If the luminescent substance emits a quantum within  $10^{-8}$  sec to a few microseconds of the time of activation by an ultraviolet quantum or a high-speed electron or ion, the process is called "fluorescence." If, however, there is an appreciable delay in the reemission of quanta, the process is known as phosphorescence. Many of the phosphors used in fluorescent lamps are sufficiently phosphorescent so that there is a marked reduction in "flicker" on 50- and 60- cps a-c circuits.

Although a very large number of organic and inorganic substances are luminescent when exposed to ultraviolet flux, only a few of the inorganic compounds have the required high sensitivity at 253.7  $m\mu$ , high quantum efficiency, desirable spectral-emission characteristics, and physical and chemical stability for use in fluorescent lamps. The phosphors most frequently used are either double oxides or salts of inorganic acids, i.e., boric, silicic, phosphoric, and tungstic. The base metal is usually of magnesium, beryllium, zinc, cadmium, or calcium.

Most synthetic phosphors consist of a highly purified oxide or salt to which is added a trace of an activator, usually a heavy metal such as manganese, nickel, or silver. In the pure state many crystalline materials exhibit little photoluminescence, but minute traces of activator materials, to the extent of less than 1 part per million, will increase the luminescence by a hundredfold. The activator also controls in part the region of maximum sensitivity and the spectral energy distribution.

#### SPECTRUM

The complete emission spectra of some typical fluorescent lamps are given in Fig. 3-15. The "white" fluorescent lamps employ combinations of two or more phosphors. By controlling the proportions of blue-, green-, and red-emitting phosphors, various colors of "white" radiation can be obtained. The complete emission spectrum of the fluorescent lamp consists of the phosphor emission upon which is superposed a weak line spectrum of the mercury discharge.

#### COLOR TEMPERATURE

The color of "white" fluorescent lamps is characterized by the temperature of a complete radiator having the closest color match. Since the spectrum of mixtures of various phosphors never produces a spectral energy distribution that even closely approximates the Planckian radiation curve, color temperature is useful only as a means of approximate color specification. The white lamps contain phosphor combinations that emit radiant energy approximating color temperatures from 3500° to 6500°K. The 3500°K lamp approximates the color of incandescent-lamp radiation, and the 6500°K lamp approximates sun and sky radiation.

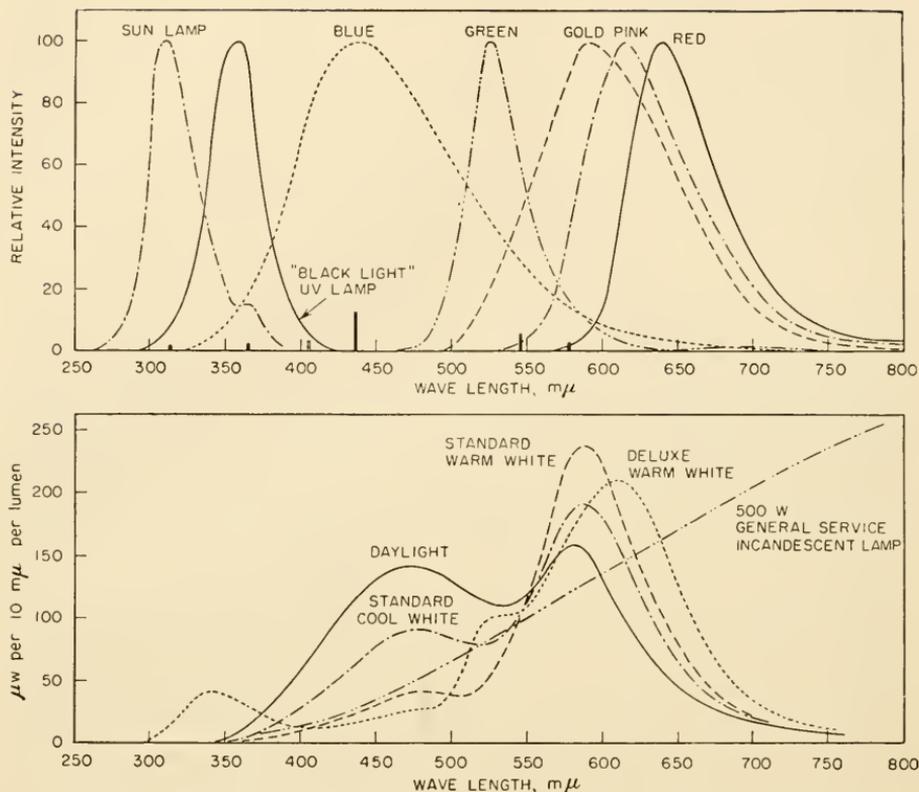


FIG. 3-15. Spectral emission of fluorescent lamps. The data include only the fluorescence spectra from the middle part of the tube. The weak line spectra from the mercury discharge and the infrared radiated by the filaments are not included in the curves. The relative intensities of the mercury lines are indicated by the black bars on the upper graph. (Data by courtesy of General Electric Company.)

#### LUMINOUS EFFICIENCY

The luminous efficiency of the radiant energy of the phosphors of the standard commercial "white" fluorescent lamp is over 300 lumens  $w^{-1}$ , which is nearly half the theoretical efficiency of a yellow-green source with all its energy at 555  $m\mu$ . This high efficiency implies that the phosphor radiant energy is confined largely to the visible and that relatively little is radiated in the infrared and ultraviolet. However, in the complete lamp, infrared from the electrodes and the warm tube reduces the luminous efficiency to about 150 lumens  $w^{-1}$ . The fluorescent lamp has been consequently characterized as a "cool" source. A 40-w fluorescent lamp converts about 20 per cent of the input electrical power into the visible and over 25 per cent into the infrared (Table 3-13). Most of the remaining power is dissipated as heat to the surrounding air by conduction and convection. By contrast, the incandescent lamp radiates over 90 per cent of the input electrical power.

For equal power input all sources will add the same thermal load to a constant-temperature room, but the fluorescent lamp tends to heat directly the air surrounding it, whereas the incandescent lamp produces most of its heating effect upon the objects incident to the flux. For this reason the radiation from a fluorescent lamp "feels cooler" than that of

TABLE 3-13. COMPARATIVE SPECTRAL CHARACTERISTICS OF SOURCES

Source	Power, w	Percentage of input lamp watts radiated in each wave band							Percentage of total radiated
		0-280	280-320	320-380	380-500	500-600	600-760	760-∞	
		mμ	mμ	mμ	mμ	mμ	mμ	mμ	
Sun, air mass = 2.....		0	0.4	2.6	13.6	19.3	24.8	39.3	
Incandescent lamp									
General service.....	40	0	0	0.03	0.6	1.6	5.0	76	83
General service.....	500	0	0	0.09	1.2	2.7	8.0	79	91
Photoflood.....	1000	0	0.02	0.3	2.6	4.9	11.0	77	96
Mercury arc									
AH-4.....	100	0	0.7	4.3	4.6	5.6	0.2		
EH-1.....	400	0	0.02	2.5	6.0	8.5	1.1		
AH-6 (all quartz).....	1000	3.1	7.5	9.0	15.0	10.5	3.7		
UA-2 (all quartz).....	250	4.6	4.3	3.4	2.8	3.8	0.4		
Germicidal.....	30	23.0	0.5	0.4	1.4	0.7	0.04		
Fluorescent lamp									
Standard cool white.....	40	0	0.2	0.6	6	8.7	4.4		
Blue.....	40	0	0.05	1.2	16	5.5	0.4		
Green.....	40	0	0.04	0.4	3	16.0	0.3		
Pink.....	40	0	0.08	0.2	2	3.3	9.2		
Gold.....	40	0	0	0	0	6.6	4.6		
Red.....	40	0	0	0	0	0.1	2.4		
Sodium lamp									
NA 9.....	180	0	0	0.82	0	11.4	0.7		

a bare incandescent lamp and produces less heating of irradiated objects for the same visible irradiance.

The electrical efficiency of the large white fluorescent lamps in lumens per electrical watt is the highest of any source for general irradiation, varying between 50 and 65 lumens  $w^{-1}$  for the various white lamps alone and 40 to 50 lumens  $w^{-1}$  for the lamp in combination with the ballast. Because of the variation in efficiency of the different types of ballasts, manufacturers rate gaseous discharge lamps on the basis of the efficiency of the bare lamp, not including the ballast losses, which amount to 15 to 30 per cent of the total power consumed by the combination.

#### TEMPERATURE

Unlike that of the incandescent lamp, the intensity of the fluorescent lamp is markedly influenced by air temperature and the rate of air circulation about the tube. The tube temperature for maximum efficiency is about 48°C. At 30° and 60°C the intensity is between 70 and 80 per cent

of the maximum. The variation in intensity with temperature is primarily due to variation in 253.7-m $\mu$  resonance radiation in the mercury discharge. Below 48°C the discharge is deficient in mercury ions; above 48°C the efficiency of production of resonance radiant energy is decreased. Phosphor efficiency is influenced only slightly by temperature, the efficiency being highest at low temperatures. These are very important considerations when large banks of lamps are operated in enclosures.

## INTENSITY

The fluorescent lamp is inherently a low-intensity low-brightness source, the white lamps having brightness values ranging from 0.01 to 0.03 c mm<sup>-2</sup> (Table 3-14). Low brightness is an asset for general irradi-

TABLE 3-14. COMPARATIVE TOTAL RADIATION CHARACTERISTICS OF SOURCES

Source	Power, w	Bright- ness, c mm <sup>-2</sup>	Luminous efficiency, lumens w <sup>-1</sup>		Color temp., °K	Wave length of maxi- mum in- tensity, m $\mu$
			Absol- ute	Elec- trical		
Sun, air mass = 2.....	.....	1650	100	..	5000	500
Black body, 3000°K.....	.....	28	19	..	3000	1040
Incandescent lamp						
General service.....	40	4.5	17	11	2760	1020
General service.....	500	9	23	20	2960	1000
Projection.....	500	16	30	26	3190	900
Photoflood.....	1,000	30	40	36	3360	850
Tungsten arc, G.E.						
Photomicrographic.....	330	48	46	..	3700	700
Carbon arc						
Low intensity, 30 amp...	1,650	150	...	13	3600	1000
High intensity, 180 amp..	13,300	950	...	25	6000	600
Flame, rare-earth, 40 amp	1,500	.....	...	60	4700	400
Zirconium arc						
0.085-mm-diam. source..	2	96	...	..	.....	1000
1.50-mm-diam. source...	100	52	...	..	.....	1000
Xenon arc, 3.5-mm gap....	150	170	...	18	4000	1050
Mercury arc						
Low pressure, germicidal.	30	.....	...	..	.....	254
H-4, 8 atm.....	100	18	...	27	.....	365
H-6, 110 atm, water- cooled.....	1,000	300	...	65	.....	436
Fluorescent lamp						
Standard cool white, 60 in.	85	0.008	110	55	4500	590
Green, 48 in.....	40	0.01	150	75	.....	530
Sodium lamp.....	180	0.06	...	53	.....	589

ation, where it is desired to obtain uniform flux distribution over large areas. Any attempt to increase the brightness greatly by increasing the current density within the lamp is defeated in part by a marked drop in efficiency of ultraviolet production by the arc discharge.

#### CATHODES

Commercial fluorescent lamps are available with cold cathodes, as used in neon sign lighting, and with hot cathodes of the preheat and instant-or quick-starting types. In general, the instant-starting hot-cathode lamps are to be preferred to the preheat lamps. The life of the lamp is determined chiefly by the rate of depreciation of cathode emission and is therefore a complex function of the frequency of starting and the time of operation.

#### ELECTRICAL CHARACTERISTICS

Like all gaseous discharge lamps, the fluorescent lamp has a negative resistance characteristic and requires a current-limiting ballast. In addition, the ballast must supply sufficient open-circuit voltage to initiate the discharge, which is usually more than twice that required for its operation. For lamps under 30 in. in length, the ballast for alternating current is an inductance and for direct current is a resistance, since the standard 118-v service is adequate to initiate the discharge. For tubes longer than 30 in., the lamp must be operated on a higher voltage, and the ballast may be a combination transformer and limiting impedance. The use of an inductive ballast on alternating current results in a low power factor, which may be less than 50 per cent. For installations involving many lamps, the current demand on the power system may become excessive, and high-power-factor ballasts are usually employed. The two-lamp ballast corrects the power factor and greatly reduces flicker by shifting the current in the two lamps out of phase. The current of one lamp leads the line voltage while the other lags; thus the radiation of one lamp is at a maximum when the other is approaching a minimum. In the four-lamp ballast, two lamps are in series on each phase.

There are four general methods of powering large banks of fluorescent lamps for the irradiation of plants. Conventional two-lamp power-factor-corrected ballasts may be used. The principal disadvantages are that, if the ballasts are mounted in close proximity to the lamps and in the same room, provision must be made for cooling the assembly, since even the "tulamp" ballasts consume 15-25 per cent of the total power. Placing the ballasts in a separate room seriously complicates the wiring, since at least one wire is required per lamp.

A second method consists in using an incandescent lamp both as a resistive ballast and to supplement the fluorescent-lamp radiation (Parker and Borthwick, 1950). Such operation has low power efficiency and

requires the use of a 450- to 600-v power source for 8-ft lamps, and lamp life is considerably below normal.

The third method involves the high-voltage series operation of "instant-start" lamps. Very satisfactory results have been obtained by using approximately 600 v rms per 96-in. tube in series at currents of 300-600 ma. A bank of 34 tubes operating in series on 20,000 v with a single 600-ma power transformer and a separate inductance as a ballast has given excellent performance as to reliability and lumen maintenance over a 6000-hr life (Withrow and Harrison, unpublished). The principal advantage of such operation is the almost complete lack of internal wiring and the relatively high efficiency of large transformers and inductances. The transformer and choke can be placed at any convenient location, and a single two-conductor high-voltage cable is all that is needed to power the bank. The disadvantage is the personnel hazard involved in handling such high voltages in a laboratory and the extreme protective measures that must be employed.

A fourth method employs paper capacitor ballasts on a high-frequency power supply. Campbell and Bedford (1947) have shown that, at high frequencies of 300-600 cps, small and inexpensive ballasts may be used. The power dissipated in the paper capacitor ballast is negligible. For 96-in. lamps, however, the power supply must be at least 450 v rms, and preferably 600. The high frequency may be obtained from a high-frequency resonant magnetic converter (Campbell, 1948), which converts the 60-cps power frequency to some higher harmonic, such as the ninth, at 540 cps. A 400-cps motor generator set also may be used. Capacitor ballasts cannot be used at frequencies much below 400 cps for gaseous discharge lamps. At 60 cps a capacitor ballast leads to very unstable operation and a sharply peaked wave form that is destructive of cathodes.

#### SHORT-DURATION SOURCES

Sources that emit radiation in short pulses of  $10^{-8}$  to  $10^{-3}$  sec are used in many types of experimentation for the determination of the kinetics of thermochemical reactions associated with photochemical processes, in photography, and as sources for studying high-speed mechanical systems. Flash tubes have been used in studies of the kinetics of photosynthesis by several investigators. Short-duration sources may be classified as single-flash types, in which the basic element must be replaced after each flash, and as repetitive-flash types, where high speeds of flashing can be maintained for thousands of pulses before the tube must be replaced.

#### PHOTOFLASH LAMPS

This type of lamp is extensively used in photography and is a chemical source in which the radiant energy is produced by the burning of alumi-

num or magnesium in an atmosphere of oxygen. Such sources produce a continuous spectrum that is rich in visible and ultraviolet, and the flash lasts for 50–100 msec. As these lamps can produce only one intense flash, their usefulness for biological work is very limited.

#### EXPLODED WIRES

One of the most intense sources known is that of wires exploded by a high-voltage capacitor discharge. Such a discharge can produce brightness values of over  $150,000 \text{ c mm}^{-2}$  and a spectral energy distribution corresponding to a black body at  $20,000^\circ\text{K}$  (Conn, 1951). The duration is of the order of microseconds per flash. Such wires can be arranged in a magazine and fired in quick succession to produce repetitive flashes.

#### SPARKS

Repetitive flashes can be obtained by gaseous discharge sources, either as open arcs, as sparks, or as enclosed discharge lamps in various gases and metallic vapors. The open spark has the advantage of relative simplicity, easy replacement, and almost unlimited power input. This type of source is used extensively for ballistic studies of projectiles, exploding objects, and rotating machinery. Such discharges from capacitors charged to high voltages have color temperatures in the range  $6000^\circ\text{--}7000^\circ\text{K}$ . The spectral characteristics of the discharge are determined in large part by the nature of the electrodes, for which aluminum, tungsten, and various iron alloys have been used extensively (Frungel, 1948; Hollaender and Foerst, 1933; Hoyt and McCormick, 1950; Quinn and Bourque, 1951).

#### STROBOSCOPIC DISCHARGE LAMP

Practically any discharge lamp can be operated at very high intensities of short duration by discharging high-voltage capacitors through it. When used as a stroboscopic source, the tube is flashed at the frequency or a subfrequency of the speed of rotation of a mechanical system. The mechanism then appears to be stationary, and many detailed operations can be observed. The spectral characteristics of flash lamps are determined by the filling gas. Krypton, xenon, neon, and mercury vapor are frequently employed. High-voltage discharges produce a spectrum characteristic of that of a high-pressure type of arc; the spectrum is a broad continuum, usually concentrated in the visible and near ultraviolet.

Flash tubes and sparks are triggered by various methods, including a high-frequency discharge to start ionization, a third electrode placed close to a pool of mercury or other electrode for initiating ionization, or the use of grid-controlled gas-type tubes for controlling the main discharge (Bourne, 1948; Forsythe and Adams, 1948).

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### 3. CONTROL OF RADIANT ENERGY

The following discussion pertains to the general properties of the more common materials, optical components, and instruments used for controlling the spectral composition and intensity of radiant flux.

#### OPTICAL PROPERTIES OF MATERIALS

Optical materials most generally used are (1) transparent substances for lenses, prisms, diffusing screens, and windows; (2) reflecting materials

for mirrors and for producing diffuse reflection and scattering inside chambers; and (3) highly absorbing material such as low-reflectance black coatings and metallic blacks.

#### TRANSPARENT MATERIALS

The optical properties of the important transparent materials for prisms and lenses are available in several monographs on spectroscopy (Harrison *et al.*, 1948; Sawyer, 1944) and physical methods (Strong, 1943) and in the various physical and chemical handbooks. The optical properties of

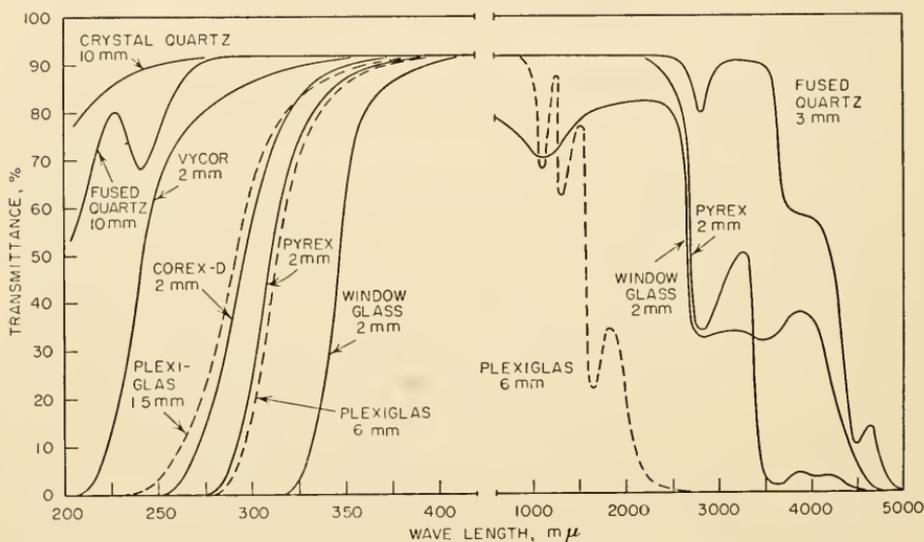


FIG. 3-16. Spectral transmission of various types of transparent materials.

halide crystals, useful in infrared spectroscopy, have been summarized by the Harshaw Chemical Company.

The most useful window materials are fused quartz (silica), the glasses, and some of the transparent plastics. Their transmittances are plotted in Fig. 3-16. The transparent plastics, especially the acrylic (Lucite and Plexiglas) and polystyrene, are relatively inert chemically, fairly resistant to continuous exposure to water, and transparent in the ultraviolet to about 300 mμ (Kremers, 1947; Wearmouth, 1943) (Fig. 3-16) and in the infrared to about 2200 mμ. Silver chloride, when grown as a synthetic single crystal, has the mechanical properties of a tough plastic and can be rolled into thin transparent films and pressed into optical components such as lenses (Harshaw Chemical Company; Kremers, 1947). When freshly prepared, it is very transparent, but its visible transmittance decreases rapidly on exposure to the visible and ultraviolet. It is especially useful as a window material for the infrared from 1 to 25 μ. Since it wets glass and metals at 200°C, infrared absorption cells and windows

resistant to all organic solvents can be constructed without the use of gaskets.

Often it is necessary to diffuse a beam so as to get uniform flux distribution over a limited area. This can be accomplished with a variety of diffusing materials such as tracing paper or sandblasted, ground, or etched surfaces of quartz or glass or by the use of pattern-molded window glass. Paper and ground glass produce much more general scattering and more reflection loss than the molded glasses. Where extreme diffusion of the beam is not required, the molded window glasses, with patterned surfaces of closely spaced small lenses or tetrahedra, are very effective and introduce relatively little energy loss in the visible region.

#### REFLECTING MATERIALS

The spectral reflectances of certain of the common metals and white pigments are plotted in Fig. 3-17. Silver and amalgamated mercury

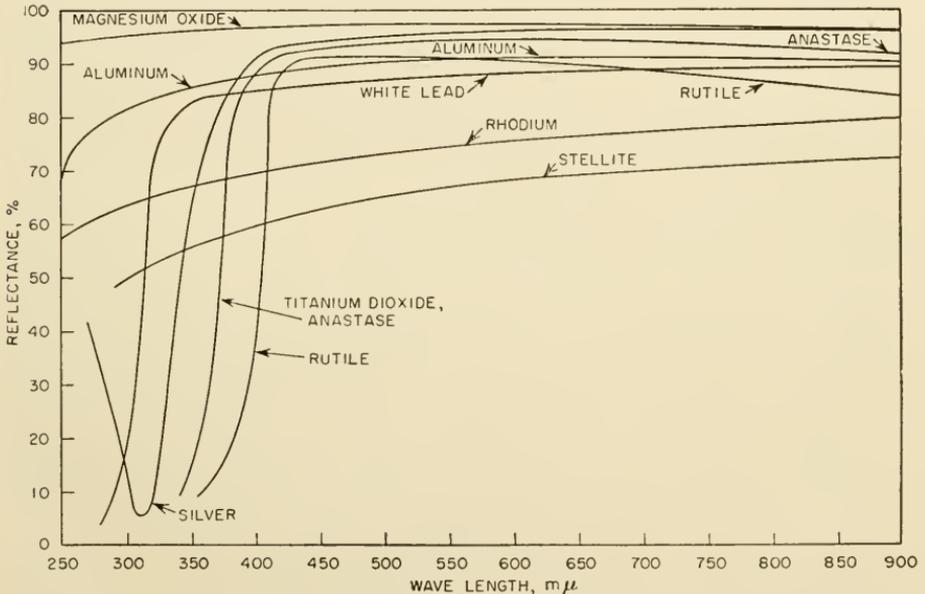


FIG. 3-17. Spectral reflection of white metals and paint pigments.

were the earliest mirror metals, but evaporated aluminum, chromium, and other metals have taken their place for critical applications. Silver has the highest reflectance in the visible and is still used for second-surface or back-silvered mirrors, but it tarnishes too rapidly for first-surface mirrors and has a narrow region of low reflection at about 310 mμ.

The front, or first-surface, mirror has the reflecting material evaporated on the front of the glass and has replaced the more expensive total-reflecting prism for many applications. Aluminum has excellent reflecting properties (Taylor and Edwards, 1931) but is soft and easily

scratched unless protected by an evaporated film of silica or other transparent coating. Evaporated films of chromium, nickel-chromium alloys, and rhodium (Claiborne, 1947; Sabine, 1939) produce surfaces that are much more scratch-resistant and have reflecting properties equivalent to those of aluminum in the visible and near ultraviolet.

"White" coatings with high diffuse reflectance throughout the spectrum are often required for the interiors of irradiation chambers and integrating, or Ulbricht, spheres. The spectral characteristics of a thick white coating depend on the spectral transmittance of the "pigment" crystals and the vehicle. The "hiding power," which is inversely proportional to the thickness required for complete opacity, primarily depends upon the refractive index of the pigment; the higher the refractive index, the thinner the layer of crystals required. Basic lead carbonate (white lead), with a refractive index of 2.1, is one of the oldest of the white pigments. The two titanium dioxide pigments have almost replaced white lead in modern finishes, partly because they are nontoxic and because both crystalline forms have a higher refractive index (2.61, rutile; 2.54, anatase). However, the titanium pigments have low ultraviolet reflectance (Judd, 1949; Mattiello, 1946) beyond 400  $m\mu$ . White lead has high visible and ultraviolet reflectance and is therefore a much more versatile pigment for the near ultraviolet. Aluminum and other metallic paints that contain a powdered white metal dispersed in a vehicle have anomalous reflection properties. Aluminum-coated surfaces never appear quite white, and yet their reflectance may approach that of surfaces coated with white crystalline pigments (Coblentz, 1912; Mattiello, 1946).

Vehicles of the drying oils and resins yellow with age, and the near-ultraviolet reflectance decreases. Therefore the most permanent whites are obtained with vehicles of the clear, colorless synthetic resins such as the polymethacrylates. These resins also impart a high degree of chemical resistance to the finish and are used in many industrial coatings. "Glossy" finishes contain a high proportion of vehicle in relation to pigment, and the surface is smooth and exhibits considerable specular reflection. Many "flat" commercial finishes contain a minimum of vehicle and a "flattening" agent that tends to produce a roughened surface. The flat finishes, especially those of the low-vehicle type, are preferable for obtaining the maximum diffusion of the incident flux, but the surface is less durable.

Magnesium oxide deposited from burning magnesium ribbon is the primary standard of white reflectance and is the coating most often used in small integrating photometer spheres (Benford *et al.*, 1948; Middleton and Sanders, 1951, 1953; National Bureau of Standards, 1939). Since the refractive index of magnesium oxide is only 1.74, a deposit several millimeters thick is required for opacity; therefore many layers of coating must be deposited on a white metal or porcelain-enameled surface. A

silver-plated and polished surface smoked five times with burning magnesium ribbon has a reflectance of over 97 per cent throughout the visible and is still above 94 per cent at 250  $m\mu$  (Middleton and Sanders, 1951). Aging and ultraviolet irradiation cause the surface gradually to lose its ultraviolet reflectance and to become slightly yellow. Magnesium carbonate has nearly as high a reflectance as the oxide and is more stable but must be deposited in a paint vehicle or used as solid blocks for a white standard. Middleton and Sanders (1953) have described a barium sulfate paint using methyl cellulose as a vehicle that is nearly as effective as magnesium oxide for spheres and much more durable.

#### BLACK COATINGS

There are three classes of "black," or highly absorbing, coatings: (1) those depending upon absorbing pigments such as carbon and the black metallic oxides; (2) evaporated or sputtered metallic blacks; and (3) black organic dyes. Carbon is unusual as an absorbing material in that it has very high and uniform absorption throughout the ultraviolet, visible, and near and middle infrared (Coblentz, 1912). The oxides of iron and copper are not so uniformly black throughout the spectrum. The base metals used in the fabrication of instruments, such as iron and steel, and copper, brass, and bronze can be blackened with a tenacious black film of oxides by the use of suitable alkaline oxidizing solutions.

Small objects, as the targets of thermocouples and bolometer strips, are blackened by the evaporation or sputtering of various metals and certain sulfides in a vacuum (Strong, 1943). Extremely thin films can be produced which absorb 95 per cent or more of the incident energy throughout the ultraviolet, visible, and infrared. The high absorption is due to extremely fine needle-shaped crystals so closely spaced that the radiant energy is trapped by multiple internal reflections between adjacent crystals. Platinum black is produced when platinum is deposited electrolytically in the presence of a trace of lead (Britton, 1951).

By the proper choice of visible and ultraviolet absorbing dyes or stains, fabrics and dyed films of gelatin and other plastics can be made completely absorbing in the visible and ultraviolet. However, organic dyes transmit freely in the near infrared; some so-called "black" dyes begin to transmit at 700  $m\mu$ . Such materials cannot be considered as opaque for any investigation involving photoprocesses whose action spectra extend into the near infrared.

#### SPECTRAL CONTROL OF RADIANT FLUX

Modification of the spectral energy distribution of the source may involve the use of a simple water cell to remove the infrared or, at the other extreme, a double monochromator to obtain the ultimate in spectral resolution and purity. Regardless of the method used, a compromise is

imposed between the two incompatible factors of radiant power and spectral resolution. High irradiances over large areas are attainable with simple solution and gelatin or glass-filter systems in conjunction with both concentrated and distributed sources, but the spectral resolution is usually limited to hundreds of millimicrons for the visible and near ultraviolet, except at the few wave lengths where such sources as the mercury arc have intense and well-isolated lines. With single interference filters, one is limited to much lower values of total flux owing to the small size of these filters and the collimation requirements, but the spectral resolution is 5–50  $m\mu$ . With the spectrograph or monochromator, the source requirements become still more stringent, and a smaller proportion of the source power can be used, but the spectral resolution can be extended well below 1  $m\mu$ .

#### FILTERS

Radiant-energy filters may be divided into two general classes: the selective-absorption filters and the optical filters. The selective-absorption filters consist mainly of organic dyes and inorganic ions in solution in water or glasses, and since they require no collimation of the incident flux, both concentrated and distributed sources may be used with high efficiency. In general, the organic dyes have sharper absorption bands than aqueous solutions of inorganic salts of most of the heavy metals except the rare earths. The absorption bands of the inorganic ions become still less sharp when incorporated in glass. The absorption bands of dyes and inorganic ions are principally due to electronic resonance phenomena in which the absorbed energy is eventually degraded to heat within the filter, and means must be provided for dissipating that energy. In the optical filter, selective interference or scattering causes the desired region to be passed by reflection or transmission, and the unwanted regions are deviated from the main beam by transmission, reflection, or scattering. Although the optical filters have the sharper and more versatile transmission bands of the two classes of filters, collimated optical systems involving concentrated sources are required, and there is more background transmission or "leakage" throughout the whole spectrum.

Insufficient attention has been given to the general or background radiant flux transmitted in the nonpass region. Published data seldom give an adequate picture of the magnitude of this background. Direct photometric measurements usually become uncertain at transmittance values much below 1 per cent, although a transmittance of  $10^{-4}$  per cent may be significant when, for example, one is working at the limit of an action spectrum curve.

*Selective-absorption Filters.* Selective-absorption filters for the isolation of narrow spectral regions have been described in various publications (Corning Glass Works; Davis and Gibson, 1931, 1934; Eastman Kodak

Company, 1951; Harrison *et al.*, 1948; Jena Glass Works; Jones, 1930; Kasha, 1948; Withrow and Price, 1953).

1. Water and inorganic salt solutions. Water (Curcio and Petty, 1951) and the inorganic solutions of ferrous ammonium sulfate and copper sulfate (Kasha, 1948; Pfund, 1939; Withrow and Price, 1953) are the most useful filters for absorbing the infrared, especially with high-power sources. One centimeter of water removes practically all the infrared beyond 1400  $m\mu$  (Fig. 3-18, Table 3-15), or about 75 per cent

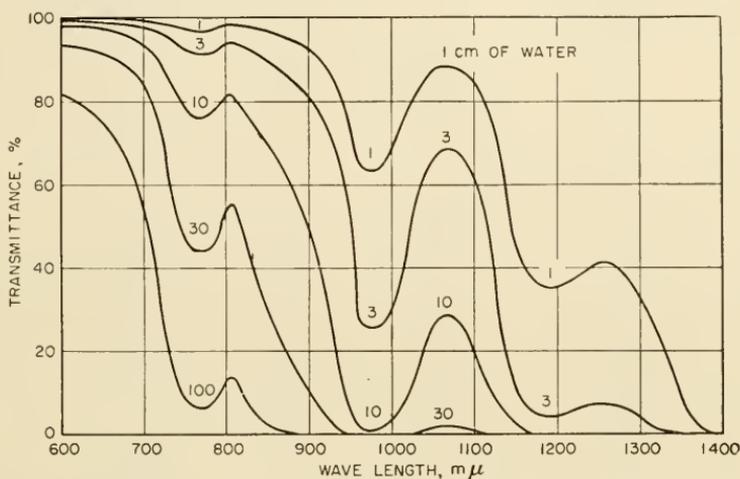


FIG. 3-18. Spectral transmission of liquid water. The numbers on the curves refer to the path length in centimeters. (Data from Curcio and Petty, 1951.)

of the total energy radiated by an incandescent lamp; a 10-cm layer absorbs all the energy beyond 1150  $m\mu$ . Ferrous ammonium sulfate in high concentrations and 10-cm layers (Fig. 3-19) removes the red and infrared. Unfortunately, ferrous ammonium sulfate is unstable and oxidizes readily to the ferric form on exposure to air. Addition of 1 per cent free sulfuric acid and a small amount of iron wire greatly retards the rate of oxidation. Copper sulfate solutions (Fig. 3-20) tend to hydrolyze and become slightly cloudy unless a small amount (0.1–0.3 per cent) of free sulfuric acid is added.

Water-filtered high-power incandescent irradiation assemblies present a difficult problem of cooling, especially for systems that are designed to irradiate a horizontal plane, as for the growing of plants. When the lamp source is mounted above a water filter, most of the energy is absorbed in the upper layers, there is little convection, and surface evaporation is rapid. Cooling coils in the body of the water do not aid in cooling the surface because of the lack of convection currents. The most effective means of cooling is to place coils of flowing tap water in the air space above the water surface so that the water vapor can be condensed back into the tanks (Withrow and Elstad, 1953).

TABLE 3-15. SPECTRAL ABSORBANCE INDEX OF LIQUID WATER AT 20°C

Wave length $\lambda$ , m $\mu$	Absorbance index $a^*$	Wave length $\lambda$ , m $\mu$	Absorbance index $a^*$
200	0.035	980	0.193
220	0.014	1000	0.159
240	0.0059	1020	0.108
260	0.0040	1040	0.0686
280	0.0033	1065†	0.0560
300	0.0028	1080	0.0569
320	0.0019	1100	0.0742
360	0.00082	1120	0.112
400	0.00035	1140	0.265
450	0.00017	1160	0.405
500†	0.00016	1185‡	0.454
550	0.00020	1200	0.443
600	0.00087	1220	0.427
650	0.00134	1240	0.402
680	0.00176	1255†	0.386
700	0.0025	1280	0.406
715	0.0039	1300	0.469
720	0.0052	1320	0.621
730	0.0074	1340	0.868
745	0.0109	1360	1.320
750	0.011	1380	2.37
760	0.011	1400	5.47
770‡	0.012	1450‡	11.25
780	0.011	1500	7.86
790	0.010	1550	4.25
805†	0.0087	1600	2.79
820	0.011	1650	2.21
840	0.016	1690†	2.18
860	0.019	1750	2.81
880	0.023	1800	3.51
900	0.031		
920	0.037		
940	0.090		
960	0.184		
970‡	0.199		

\* The absorbance index  $a$  is defined by the formula

$$ab = -\log T = -\log I/I_0,$$

where  $b$  is the path length in centimeters,  $T$  is the transmittance,  $I_0$  is the relative intensity of the incident beam, and  $I$  is the relative intensity of the transmitted beam.

† Absorption minima.

‡ Absorption maxima.

For a horizontal beam of radiant energy the conventional cylindrical filter cell with two vertical glass windows can be cooled very satisfactorily with a cooling coil in the solution. In this case the heating occurs in a vertical plane, and convection currents are induced. Acidulated copper

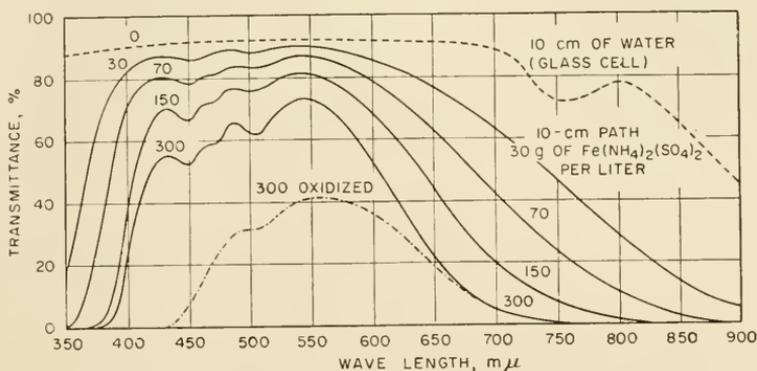


FIG. 3-19. Spectral transmission of ferrous ammonium sulfate solutions in a 10-cm path. The solutions were prepared in 2 per cent sulfuric acid at 30, 70, 150, and 300 g per liter of solution, as indicated on the solid curves. The upper dashed curve is for water; the lower dash-dot curve is for 300 g per liter of solution exposed to the air for 2 weeks. (From Withrow and Price, 1953.)

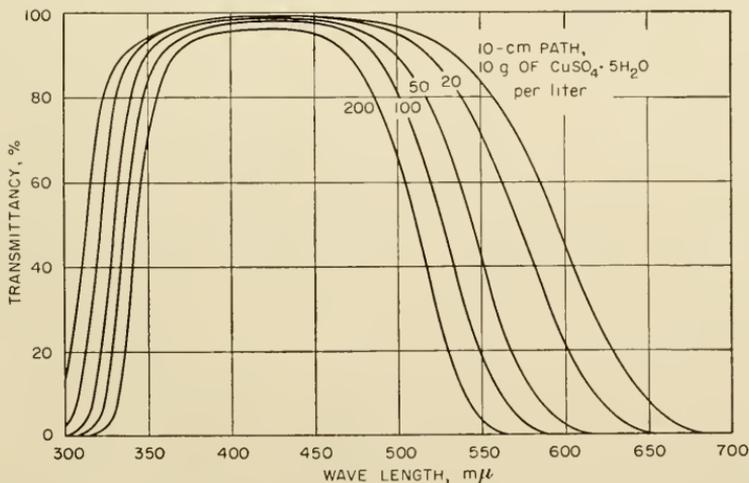


FIG. 3-20. Spectral transmission of copper sulfate solutions in a 10-cm path length at the concentrations indicated in grams per liter of solution made up in 0.5 per cent sulfuric acid. (From Withrow and Price, 1953.)

sulfate solutions corrode copper, aluminum, tin, and lead tubing, but stainless-steel, silver, or glass tubing may be used.

2. Dye solutions and films. The three most useful classes of dyes for the preparation of filters are the water- and alcohol-soluble basic, acid, and direct, or substantive, dyes. Biological stains are of higher purity

than the fabric dyes, but they are not available in so great a variety of spectral characteristics and are more expensive. The water-soluble dyes may be incorporated in water-dispersible or water-permeable plastic films of gelatin, polyvinyl alcohol, cellophane, or nylon.

Since gelatin is relatively transparent to the visible and ultraviolet to beyond  $300\text{ m}\mu$  and is water-dispersible, it forms a convenient dispersing medium for the water-soluble organic dyes. Dyed gelatin films, such as Wratten filters (Eastman Kodak Company, 1951), have been used extensively in photography and photochemical investigations. Large dyed-gelatin filters can be made in the laboratory by the direct casting of dye-gelatin solutions on glass (Withrow and Price, 1953). The alcohol-soluble dyes can be incorporated directly into organic solutions of the colorless plastics. A series of visible-absorbing, infrared-transmitting filters have been developed in this manner (Blout *et al.*, 1946; Shenk *et al.*, 1946). Infrared-transmitting filters with high thermal stability can be prepared by the thermal polymerization of plastic films in which the plastic itself becomes the filter (Blout *et al.*, 1950).

Cast sheets of the acrylic resins, such as Plexiglas or Lucite, strongly absorb the middle and far infrared. A sheet of clear plastic 5 mm thick absorbs the infrared beyond  $2500\text{ m}\mu$ ; a 2-cm sheet absorbs beyond  $1700\text{ m}\mu$ ; ultraviolet absorption begins at about  $290\text{ m}\mu$ . There is no appreciable absorption in these plastics between 320 and  $900\text{ m}\mu$ . Because they are thermoplastic, they cannot be used where high-power dissipation is required, but they are very useful for protecting thermal detectors from long-wave-length infrared emitted by warm objects.

3. Glass filters. In the infrared and ultraviolet, certain of the glass filters (Coblentz and Stair, 1929; Corning Glass Works; Jena Glass Works; Stair, 1948; Stair *et al.*, 1949) have spectral characteristics that cannot be duplicated with dyes. This is especially true of the infrared-absorbing Aklo type and the ultraviolet-transmitting filters. The glass filters are particularly useful where permanence and heat resistance are prime considerations.

*Optical Filters.* The optical filters have greater spectral range and resolution than the selective-absorption filters, but they all require some degree of beam collimation; thus only high-intensity concentrated sources can be used. The optical systems may be quite simple, since an appreciable beam divergence can be tolerated without seriously widening the transmission band, and a projection-lamp filament at the focus of a simple plano-convex lens is usually adequate.

1. Christiansen filter. The Christiansen filter consists of a transparent cuvette containing small particles of a transparent solid in a liquid. The solid and liquid are so chosen that a wave-length plot of their refractive indexes yields two curves that cross at one point where the refractive indexes are the same (McAlister, 1935; Minkoff and Gaydon, 1946;

Sinsheimer and Loofbourow, 1947). The radiant flux is scattered from the beam except at those wave lengths where the solid and liquid have the same refractive index; radiant flux of this wave length passes through the cell undeviated, and the mixture appears optically clear. At other wave lengths the degree of scattering increases with the difference in refractive index between solid and liquid. Since a small part of the scattered energy always appears in the main beam, the general background transmission is appreciable. By using several filters in tandem, with sufficient separation between them, the background can be reduced to a low value. The refractive indexes of organic liquids have high temperature coefficients; consequently the temperature must be controlled very precisely. The region of maximum transmission can be shifted slightly by changes in temperature; large changes in wave length are obtained by selecting different solid and liquid mixtures.

A very simple selective-scattering filter is used in infrared spectroscopy to scatter visible and near-infrared energy out of the main beam. A layer of very small particles of a transparent material such as powdered quartz or zinc sulfide (Henry, 1948) on the surface of a rock-salt window will scatter the shorter wave lengths but have little effect upon energy of wave lengths much longer than the dimensions of the particles. A diffraction grating can be used in a similar manner (White, 1947).

2. Interference filter. Wave interference within thin films of optical elements forms the basis for the selective spectral characteristics of the interference filter. Energy absorption within the filter components plays no constructive role in determining the spectral properties; it serves only to attenuate the peak transmission or reflection. Since the undesired regions are removed by reflection or transmission and are not directly absorbed by the filter, heating from high-intensity sources is often less of a problem than with the selective absorption filters.

The four most common types of interference filters include (1) the Fabry-Pérot etalon filter of alternate dielectric and semitransparent metal films, (2) the single or multiple dielectric-film filter, (3) the frustrated total-reflection filter, and (4) the Lyot birefringent filter. All these have been reviewed by Greenland (1952) and Turner (1950).

The Fabry-Pérot filter consists of a sandwich of two semitransparent films of metal, usually silver, separated by a thin film of a dielectric such as magnesium fluoride (Fig. 3-21). It derives its name from the Fabry-Pérot etalon interferometer, which consists of partially reflecting silver mirrors separated by a thin air space, and the same basic equations apply to both the interferometer and the filter. To form the filter, a silver or aluminum semitransparent film is evaporated in a vacuum on a plane glass surface. Then a transparent dielectric film is deposited, followed by a second transparent metal film. A protective cover glass is next cemented on the surface. The thickness of the dielectric spacer film must

be controlled with extreme care, since its thickness determines the wave length of constructive interference and maximum transmission.

The Fabry-Pérot filters have several transmission bands representing the various orders of interference (Fig. 3-21). If the first-order band occurs in the red, a second order will appear in the blue, and a third in the ultraviolet. The higher orders are readily removed by long-wave-pass dyed-gelatin or glass filters. The transmittance of the background

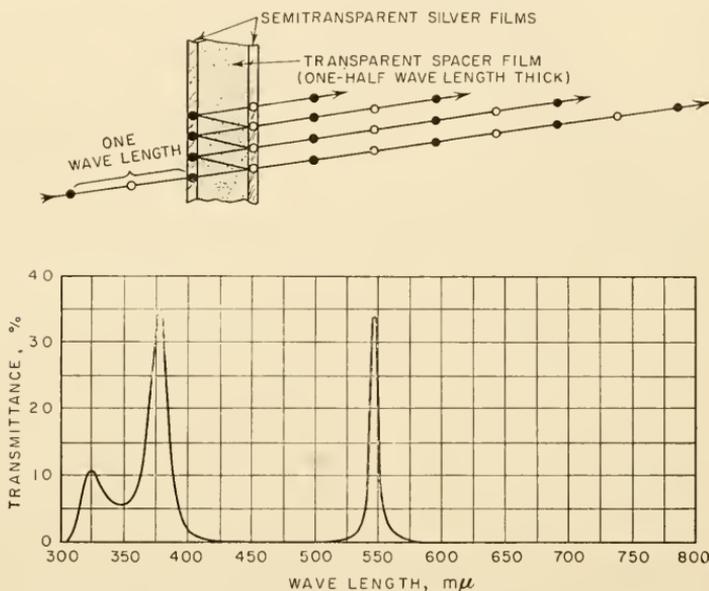


FIG. 3-21. Simplified diagram of a transmission interference filter of the Fabry-Pérot type (above) and a transmission spectrum of a second-order 546- $m\mu$  filter (below). The third- and fourth-order bands in the near ultraviolet must be removed with a yellow glass or gelatin filter. (From Turner, 1950.)

is determined by the thickness of the metal films—the thinner the film, the higher the peak transmittance and also the background. For background transmittances of the order of a few tenths of 1 per cent, the peak transmittance is usually between 10 and 40 per cent. The resolution of interference filters is specified in terms of a nominal spectral band width measured at half the peak transmittance. For many applications the effective width is several times this value. Commercial filters have nominal band widths varying from 5 to 30  $m\mu$ . By placing matched filters in tandem, the band width and background transmittance decrease more rapidly than the peak transmittance. By tilting the filter the wave length of maximum transmittance can be shifted over a range of several millimicrons. As a general rule, the incident beam must be collimated to within  $10^{\circ}$ – $15^{\circ}$  on each side of the normal to avoid excessive widening

of the transmission band (Bausch & Lomb Optical Co., 1953; Buc and Stearns, 1950; Greenland, 1952; Greenland and Billington, 1950).

The reflection filter consists of two or three metal films separated by dielectric films, the first metal film on the glass forming a total-reflecting mirror. The others are semitransparent, as in the case of the transmission filter. These filters have characteristics similar to those of the transmission filter.

The dielectric-film filters yield band-pass filters, neutral filters, and beam splitters. One or more films of transparent dielectric materials, such as magnesium fluoride or zinc sulfide, are evaporated onto the optical surfaces. Single films of thicknesses sufficient, in relation to the wave length, to produce interference of the reflected rays are used as anti-reflection coatings on optical components, such as telescope and camera lenses.

Multiple films of appropriate thickness and refractive index produce band-pass filters of high peak transmittance. They are especially useful for obtaining complementary colors (the transmitted ray is the complement of the reflected ray) and for removing broad regions from the radiation of the main beam. "Cold" mirrors for projection equipment can be made with high reflectance in the visible and low reflectance (high transmittance) in the infrared. They do not have to dissipate so much heat as glass filters, since the infrared is transmitted out the back of the projection lamp.

The frustrated total-reflection filter is a dielectric film deposited on the hypotenuse of a total-reflecting prism. This system eliminates the metal film of the Fabry-Pérot filter. The incident rays entering a reflecting prism are normally totally reflected at the hypotenuse if they are incident at an angle greater than the critical angle. If a dielectric film with a high refractive index and of the right thickness is deposited on the reflecting surface, the rays penetrate into this layer and are partially transmitted instead of being reflected; hence the name "frustrated" total-reflection filter (Billings and Pittman, 1949). These filters can be designed for any spectral region where transparent materials are available.

The birefringence, or polarization, filter was developed for the study of solar prominences, where a very narrow band pass of less than an angstrom unit was needed to isolate the hydrogen line of the prominence from the continuous background of the solar disk. This filter is the most selective of all filter systems. A birefringent, or double-refracting, crystal placed between crossed polarizers produces a "channel" spectrum in which evenly spaced bands of wave lengths are missing. By the proper selection of a series of alternate birefringent plates and polarizers, all but a few widely separated pass bands are canceled out. These filters consist of many expensive precision components and require precise collima-

tion and temperature control. Consequently their use has been limited principally to astronomical investigations (Billings, 1947). Such filters can be tuned electrically with Kerr-cell birefringent components and the band pass shifted rapidly through a limited portion of the spectrum.

#### SPECTROSCOPIC METHODS

The most versatile instrument for the isolation of narrow spectral regions is the spectroscope, which is any device capable of producing a spectrum. It usually consists of three basic components: a collimator comprised of a slit and lens or mirror for producing parallel rays, a dispersing element such as a prism or grating, and a focusing system for producing spectral images of the entrance slit. If the spectrum is imaged on a photographic emulsion or other surface, it becomes a spectrograph; if a second slit is interposed in the spectrum, thus isolating a single narrow band of wave lengths, it becomes a monochromator. Since the design and use of such instruments have been extensively covered by several monographs and papers on spectroscopy (French *et al.*, 1947; Harrison *et al.*, 1948; Sawyer, 1944) and spectrophotometry (Brode, 1943; Cary and Beckman, 1941; Lothian, 1949; Mellon, 1950; Walsh, 1952; Williams, 1948; Zscheile, 1947), only a few of the more important considerations pertaining to the selection and use of irradiation spectroscopes will be discussed.

*Dispersing Elements.* The dispersing element of the spectroscope bends the incoming rays through various angles, depending upon the wave length. This is quantitatively expressed as the angular dispersion  $d\theta/d\lambda$ , where  $d\theta$  is the increment of angular deviation in radians corresponding to the wave-length interval  $d\lambda$  (Fig. 3-22). A more directly useful quantity is the linear dispersion or the linear separation of small increments of wave length in the focal curve of the spectrum. It is principally a function of the angular dispersion, the focal length of the focusing lens or mirror, and the inclination of the focal curve. The resolving power of a spectroscope is defined as  $\lambda/d\lambda$  and is the wave-length interval between two adjacent lines which can just be resolved with the narrowest slits.

1. Dispersing prism. The dispersing prism of the spectroscope is a refracting element in which use is made of the variations in refractive index with wave length. Thus rays of different wave lengths are bent through different angles in passing through a prism. For minimum deviation in a simple prism (Fig. 3-22a) the angular dispersion is related approximately to the change in refractive index with wave length  $dn/d\lambda$  as follows:

$$\frac{d\theta}{d\lambda} = \frac{dn}{d\lambda} \cdot \frac{2 \tan i}{n}, \quad (3-19)$$

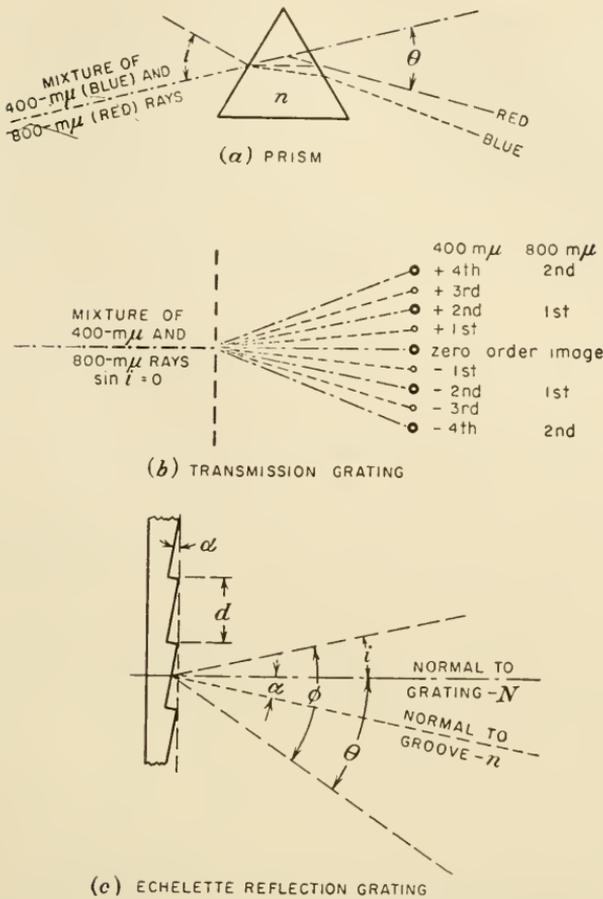


FIG. 3-22. (a) The 60° prism, (b) the transmission grating, and (c) the echelette reflection grating as dispersive elements. The angles  $i$  and  $\theta$  refer to angles of incidence and refraction (a) or diffraction (c), respectively. Diagram a shows how blue and red rays are refracted and dispersed in passing through a prism. Diagram b, for the diffraction grating, shows how the various orders of blue and red flux are diffracted on both sides of the zero-order or undiffracted image. In the echelette grating, shown in c, the most intense portion of the spectrum (the blaze) is obtained when the bottom of the groove is tilted at the groove angle  $\alpha$  so that the angle of groove incidence ( $i + \alpha$ ) of the rays to the bottom of the groove is equal to the angle of reflection ( $\theta - \alpha$ ). In the region of the blaze the rays are reflected from the bottom of the groove as in a simple mirror. The grating spacing is  $d$ .

where  $i$  is the angle of incidence at minimum deviation and  $n$  is the refractive index. The quantity  $dn/d\lambda$  is a complex function of wave length, the form of which varies greatly with different materials.

The most common prism materials for the visible and ultraviolet are glass and quartz, and for the infrared, the halide crystals, such as sodium chloride and potassium fluoride.

In the visible and adjacent regions the refractive index for most materials increases with decreasing wave length, and therefore the shortest wave lengths are most deviated. A plot of the refractive index against wave length shows that the slope of the curve  $dn/d\lambda$  decreases with increasing wave length. As a result, the dispersion  $d\theta/d\lambda$  is not uniform, and the longer wave lengths usually are relatively crowded as compared with the shorter. For common prism materials the dispersion may be ten times as great at one end of the useful range as at the other. This lack of uniform dispersion is one of the principal limitations of the prism spectroscope.

Liquid prisms have been the subject of much interest for large-aperture irradiation monochromators, since large dimensions can be obtained inexpensively. However, they have not been extensively used because of inhomogeneities caused by convection currents and the high temperature coefficients of refraction (Sawyer, 1944) as compared with those of solids. Harrison (1934) has described a reflecting liquid-prism monochromator consisting of a concave mirror immersed at an angle in a tray of water. This arrangement eliminates vertical windows and offers some possibilities for obtaining high irradiances.

2. Diffraction grating. The diffraction grating consists of very fine and closely spaced lines, ruled on an optical surface, which behave as slits in transmission gratings and line mirrors in reflection gratings. Diffraction of the radiant energy by the rulings produces a series of overlapping spectra by interference. Ideally the lines are shallow V-shaped grooves formed by a ruling engine drawing a diamond point over the surface (Fig. 3-22*b*). Early transmission gratings were ruled on hard surfaces such as glass and metal, but these materials rapidly eroded the ruling diamond. The development of the vacuum-evaporated aluminum mirror, with its soft surface and high reflection efficiency throughout the spectrum, made possible reflection gratings of large size and precisely controlled groove contour.

Standard rulings for the visible and ultraviolet are nominally 7500, 15,000, and 30,000 lines per inch (300, 600, and 1200 lines per millimeter); coarser rulings are used in the infrared. Plane gratings are ruled on optically flat aluminized mirrors and are available commercially with a ruled area of 10–20 cm square, and much larger plane gratings are in prospect. The concave grating is made by ruling on a long-focal-length aluminized parabolic mirror. Because of factors concerned with astigmatism, the lines are usually short, not over 5 cm long, although the ruled area may be much longer. Consequently the concave grating has a smaller ruled area than a plane grating with the same over-all dimensions.

Because of the great cost of producing original gratings, various methods of transferring the ruling contour of original gratings to other optical surfaces have been developed. These duplicates are known as

“replica gratings” and are available in both transmission and reflection gratings at a fraction of the cost of the originals. Modern replica reflection gratings are practically as good as the originals for monochromator use.

The angular position of the various wave lengths diffracted by a grating are given by the equation

$$m\lambda = d(\sin i \pm \sin \theta), \quad (3-20)$$

where  $m$  is the order of the spectrum on the right or left side of the central, or zero-order, image, in integers of 1, 2, 3, etc., and  $d$  is the grating spacing or the distance from the center of one line to the center of the next. For a 600-line-per-millimeter grating,  $d$  equals  $\frac{1}{600}$  mm per line. The angles  $i$  and  $\theta$  are the angles of incidence and diffraction, respectively, measured from a normal to the grating surface. This equation is valid for both transmission and reflection gratings and for all values of  $i$  and  $\theta$ . In the reflection grating,  $\sin i$  and  $\sin \theta$  will have opposite signs if they are on opposite sides of a normal to the grating surface; they will have the same sign if on the same side of the normal. It will be noted that, in contrast to a prism, the shortest wave lengths are deviated the least by a grating.

By differentiating the grating formula and keeping  $i$  constant, an expression for the angular dispersion is obtained:

$$\frac{d\theta}{d\lambda} = \frac{m}{d \cos \theta}. \quad (3-21)$$

The dispersion is proportional to the number of lines per millimeter ( $1/d$ ) and the order of the spectrum  $m$ . It is a minimum when  $\theta = 0$ , i.e., when the spectrum is observed normal to the grating. For the so-called “normal spectrum” the dispersion is approximately constant for small changes in wave length, and  $d\theta/d\lambda = m/d$ . For a grating with 600 lines per millimeter, the first-order normal dispersion is  $600 \times 10^{-6}$  radian/ $m\mu$ , since there are  $10^6$   $m\mu$ /mm. This amounts to

$$1 \times 57.3 \times 600 \times 10^{-6} = 3.4 \times 10^{-2} \text{ }^\circ/m\mu.$$

The linear dispersion  $dl/d\lambda$  for the normal spectrum of a grating is

$$\frac{dl}{d\lambda} = r \frac{d\theta}{d\lambda}, \quad (3-22)$$

where  $r$  is the distance from the grating to the focal curve of the spectrum. At 2-m distance the linear dispersion of the first order for this grating would be 1.2 mm/ $m\mu$ . Thus two lines differing in wave length by 1  $m\mu$  would appear as two lines 1.2  $m\mu$  apart.

With early gratings much of the energy was dissipated into undesired orders, since only the spectrum of one order and on one side could be

used at one time. Wood showed that, by making the sides of the groove of a reflection grating smooth and by controlling the angle of one side so that each ruling behaved as an elementary line mirror, it was possible to obtain a grating with most of the energy in one order and on one side of the central image. This type of grating he called an "echelette" (Babcock, 1944; Stamm and Whalen, 1946), and it is used in nearly all modern gratings. The high efficiency obtains for only a certain range of wave lengths or blaze where the angle of the groove, or blaze angle, is such as to cause specular reflection. Since the groove angle determines the wave length of the blaze, or the most intense portion of the spectrum, it is important to specify the region where maximum efficiency is desired when ordering a blazed grating. For example, one commercial grating of  $10 \times 10$  cm with 600 lines per millimeter and in a Littrow mounting has the following efficiencies for different wave lengths in the first order on one side: 254  $m\mu$ , 62 per cent; 265  $m\mu$ , 72 per cent; 313  $m\mu$ , 48 per cent; and 546  $m\mu$ , 18 per cent. It is evident that this grating is most useful in the ultraviolet.

The groove angle  $\alpha$  required to produce the maximum intensity at the blaze wave length can be calculated from simple geometrical analysis, considering the bottom of each groove or line as an elementary mirror inclined at the angle  $\alpha$  to the grating surface (Babcock, 1944). The incident ray and diffracted ray of the blaze wave length must make equal angles with a normal to the bottom of the groove for specular reflection and maximum energy transfer. The following relations then hold:

$$m\lambda = d(\sin i \pm \sin \theta),$$

which is Eq. (3-20), and

$$\alpha = (i \pm \theta)/2. \quad (3-23)$$

The upper signs apply when the incident and diffracted rays are on the same side of the grating normal, and the lower signs when they are on opposite sides. From these two formulas the value of the groove angle necessary for any value of  $\lambda$  and  $i$  or  $\theta$  can be calculated. It will be noted that the values of  $i$  and  $\theta$  as determined by the optical arrangement or mounting have only a small effect on the value of  $\alpha$ .

In order to cover the ultraviolet and visible at high efficiency with echelette gratings, several gratings must be available with the same grating spacing  $d$  but different groove angles  $\alpha$ . The grating previously mentioned as having the blaze at 265  $m\mu$  was designed for a Littrow mounting in which  $i = \theta$  and the incident and diffracted rays were on the same side of the normal. For this condition  $\alpha = 4^\circ 33'$ , as predicted by Eq. (3-23). Maximum efficiency at 400  $m\mu$  for the same arrangement requires a groove angle of  $6^\circ 54'$ , and for 600  $m\mu$  an angle of  $10^\circ 22'$ . However, if an incandescent source is used, the groove angle should be

selected for the shortest wave length to be used. Then the decreasing efficiency with wave length will in part compensate for the increase in spectral intensity of the source.

It is evident from Eq. (3-19) that, in the grating monochromator, energy of wave length  $\lambda$  emerging from the exit slit for the first order,  $m = 1$ , will be mixed with energy of the second order,  $m = 2$  of  $1/2\lambda$ ; for the third order,  $m = 3$  of  $1/3\lambda$ , etc. When using the first-order spectrum of a grating, it is relatively simple to eliminate the higher orders in the visible and ultraviolet with long-wave-pass glass or gelatin filters. Quartz, which absorbs all wave lengths beyond  $180\text{ m}\mu$ , will eliminate orders higher than the first to about  $350\text{ m}\mu$ ; window glass ( $300\text{ m}\mu$ ), to  $600\text{ m}\mu$ ; and yellow glass or gelatin filters ( $600\text{ m}\mu$ ), to  $1200\text{ m}\mu$ . It is more difficult to make use of the second-order spectrum and eliminate the first, third, and higher orders because band-pass filters are required.

#### *Collimator and Focusing Systems.*

Both the collimator and focusing systems can employ either positive lenses or concave mirrors. Small spectroscopes for the visible usually employ compound lenses corrected for spherical and chromatic aberration. In the ultraviolet, achromatic lenses are less commonly employed, since they are difficult to make and expensive.

For instruments of large aperture, mirror optics offer many advantages

over lenses. Mirrors introduce no chromatic aberration, and off-axis parabolic mirrors (Fig. 3-23) introduce little astigmatism and spherical aberration. Mirrors are, as a rule, less expensive than lenses for the same size and degree of correction. The two principal disadvantages of mirrors are that, since it is usually necessary that the beam be doubled back on itself, the optical arrangement sometimes presents difficult problems of

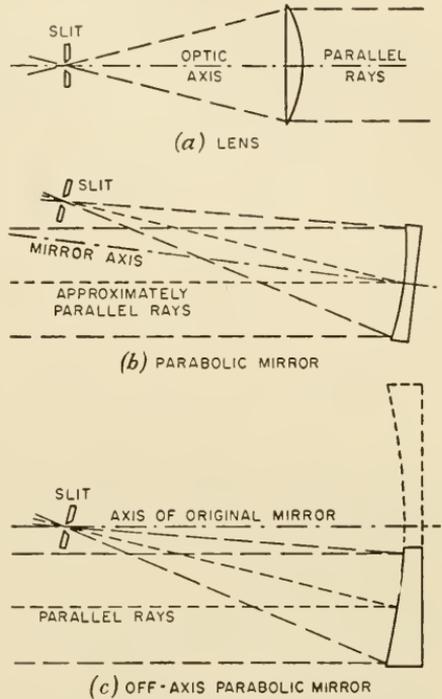


FIG. 3-23. Collimating systems employing lenses and mirrors. The slit is at the focus of the lens or mirror. The lens collimator (a) can be made approximately achromatic by the use of compound lenses; concave mirrors are achromatic. The parabolic mirror at b produces only approximately parallel rays, since the slit is not on the geometrical axis. The off-axis parabolic mirror of c is cut from a larger mirror, as indicated, and eliminates the astigmatism produced by the b arrangement.

spacing the components, and first-surface mirrors are much more easily damaged by careless handling and corrosive atmospheres than lenses. However, the fragile surfaces of aluminized mirrors can be partially protected by evaporating a protective film on the aluminum.

The effective aperture area of a spectroscopy is the area of the beam as it traverses the dispersing system. This beam is usually square or rectangular in cross section and often smaller in area than the circular aperture of the collimator or focusing optics. Various methods may be used for expressing the aperture diameter, or linear aperture, but for most practical purposes it is expressed as the diameter  $d_e$  of a circle of an area equivalent to that of the used portion of the prism or grating. The  $f$ /number, or aperture ratio, then becomes  $f/d_e$ . This quantity is usually greater than that of the collimator or focusing optics.

*Condensing Systems.* The application of the lens formula, Eq. (3-6), shows that, if the condenser lens or mirror is of the same size as the collimator, its focal length must be less. If the image magnification of the source is 1, the condenser focal length must be half that of the collimator in order to subtend the same angle as the collimator. It can be shown (Sawyer, 1944) that for a perfect optical system the image of the source is never brighter than that of the source itself. Both lenses and mirrors can be used as condensers, but mirrors often necessitate a crowded arrangement of source and entrance slit. Advantage can often be taken of the chromatic aberration of a lens to compensate partially for non-uniformity of the spectral energy distribution of the source and the power transmission of the monochromator.

*Relative Power Transmission.* The most useful criterion for the evaluation of an irradiation monochromator is the relative power  $P$  transmitted per unit source intensity  $N$  within a wave-length interval  $\Delta\lambda$ . For simplicity, consider a symmetrical monochromator with collimator and focusing systems of equal aperture area  $A$  and focal length  $f$  and entrance and exit slits each of width  $s$  and length  $l$ . Let all transmission losses due to vignetting or diaphragming or to absorption in lenses and prisms, and all reflection losses due to gratings and mirrors be included in the one coefficient  $T$ , which may be treated as an over-all transmittance. Assume that the source has a radiance of  $N$  w steradian<sup>-1</sup> cm<sup>-2</sup> and is sufficiently large so that a condensing lens or mirror can be chosen which will produce an image covering the slit. The angular aperture  $\omega$  of the condensing system is assumed to be equal to that of the collimator. The radiant power, in watts, entering the slit is then

$$P_{\Delta\lambda} = N_{\Delta\lambda} T s l \omega. \quad (3-24)$$

The allowable width of slit  $s$  for a given  $\Delta\lambda$  is a function of focal length  $f$  and dispersion  $d\theta/d\lambda$ ; then  $s = \Delta\lambda f(d\theta/d\lambda)$ . The slit length  $l$  is a design factor that is likewise proportional to the focal length:  $l = Kf$ . The

solid angle  $\omega$  subtended by the collimator is given approximately by  $\omega = A/f^2$  radians, where  $A$  is the effective aperture area. Inserting these quantities in Eq. (3-24) gives

$$P_{\Delta\lambda} = N_{\Delta\lambda} T \Delta\lambda f(d\theta/d\lambda) K f(A/f^2) = N_{\Delta\lambda} T \Delta\lambda (d\theta/d\lambda) K A$$

and

$$(P/N)_{\Delta\lambda} = K T A (d\theta/d\lambda) \Delta\lambda. \quad (3-25)$$

Thus the relative power transmitted in a specified wave-length band per unit source intensity is proportional only to the product of the transmittance, the effective aperture area, and the angular dispersion of the dispersing system and is independent of the aperture ratio.

The irradiance or intensity at the exit slit is the power per unit slit area  $sl$ ; therefore

$$\left(\frac{H}{N}\right)_{\Delta\lambda} = \left(\frac{P}{slN}\right)_{\Delta\lambda} = T\omega = T \frac{A}{f^2} = T \frac{\pi}{4} \left(\frac{d}{f}\right)^2. \quad (3-26)$$

The spectrographic speed or irradiance at the exit slit is proportional to the angular aperture and inversely proportional to the square of the aperture ratio, or  $f$ / number,  $f/d$ .

1. Aperture ratio. There has been considerable overemphasis on the importance of large angular apertures or small values of the aperture ratio in regard to irradiation and photometric monochromators. From the preceding analysis of spectroscope transmission, it is evident that, for a given source intensity and on the assumption that an adequate condensing system is available, the transmitted radiant power within a specified wave-length band is independent of the focal length of the optics. As the collimator focal length is increased, the permissible width and length of the entrance slit may be increased proportionately to maintain the same wave-length band, but the subtended angle that has to be filled by the condenser is decreased. The result is that the useful radiant power that can be made to enter the slit is ultimately dependent only on source intensity and the transmission of the condensing system.

The same analysis can be applied to the focusing system. The total power emerging from the exit slit is independent of the focal length of the focusing lens or mirror. However, the power per unit area or irradiance at the exit slit is proportional to the angular aperture but inversely proportional to the square of the aperture ratio. When the spectroscope is used to irradiate the grains of a photographic emulsion, or a small bolometer or thermocouple target, maximum irradiance is desired, and low aperture ratios of  $f/4$  to  $f/1.5$  are of real merit. On the other hand, when biological objects are irradiated whose over-all dimensions are larger than the exit slit, a short focal length is of little value.

The principal disadvantage of lenses and mirrors of small aperture ratio and therefore relatively short focal length is that the problems of

astigmatism and spherical aberration increase rapidly with decrease in focal length. As with a camera lens, the depth of focus increases as the aperture ratio decreases, and focusing becomes correspondingly less critical. The cost of short-focal-length lenses and mirrors of adequate quality for a given instrument is usually much greater than that of long-focal-length optics of the same diameter. Another factor tending to reduce the cost of small-aperture-ratio optics is the availability of long-focal-length astronomical parabolic mirrors of excellent quality in  $f/$  numbers of 8-12.

2. Comparison of prisms and gratings. The angular dispersion of the prism may be very high in the shorter wave lengths, but it decreases rapidly with increase in wave length. However, the transmission of a prism is high throughout most of its useful range. By contrast, the dispersion of the grating is nearly constant and may greatly exceed that of a quartz or glass prism in the longer wave lengths. The effective transmission is relatively low for an echelette grating in all but the spectral region of the "blaze," where the intensity is maximal. The spectral position of the blaze is determined by the groove angle.

The slit images or lines produced by a grating are less curved than those produced by a prism. This is due mainly to the smaller angle of incidence usually employed with the grating. Thus, with a grating, longer slits may be used without having to resort to curved entrance slits to obtain straight-line images. The relatively uniform dispersion of gratings makes it practical to use interchangeable fixed slits instead of continuously variable slits, as required by the varying dispersion of prisms. This is an important advantage where water-cooled entrance slits are required. Gratings have had the reputation of scattering a relatively large proportion of undispersed energy. The aluminized replica grating is a great improvement over the speculum metal grating in this regard. A well-designed plane-reflection-grating spectroscope with reflecting optics approaches comparable prism instruments as to freedom from stray flux (Donaldson, 1952).

Quartz and glass prisms are rugged, easily cleaned, and relatively permanent. Gratings are fragile, and the aluminum is damaged by corrosive atmospheres and cannot be readily cleaned. For the same effective area, the plane replica grating is much less expensive than high-quality prisms. Consequently, for the same cost, a larger aperture area can be obtained in a grating than in a prism (Harrison *et al.*, 1948).

Harrison *et al.* (1948) and Strong (1949) have reported detailed performance data on prism and grating spectroscopes. For the biological irradiation spectroscope or single monochromator in the ultraviolet and visible, the plane reflection grating of large aperture area appears to offer many advantages over the prism. For the double monochromator, where the combined power transmitted is proportional to  $T^2$ , prism instruments

are nearly always used. The echelette grating would be suitable for a double monochromator only near the spectral region of the blaze.

*Spectroscopic Optical Arrangements.* Most spectroscopic optical arrangements or mountings employ a collimator to provide parallel flux incident to the prism or grating. The notable exceptions are the Féry prism, the concave grating mountings, and the converging flux mountings for the prism and plane grating. Several typical optical arrangements for mountings for prism and grating monochromators are diagrammatically presented in Fig. 3-24.

The principle of a single lens or mirror with converging rays on the prism or plane grating has been little used in large spectroscopes and irradiation monochromators but deserves much more attention than it has received. One such arrangement for the prism is described by Parker *et al.* (1946) and has been employed for obtaining action spectra of the photoperiodic responses in plants. This type of mounting for the plane grating was originally described by Monk (1928) and later redescribed in greater detail by Gillieson (1949). It has the advantage that the slit image can be highly magnified into a large spectrum with only one optical element. There is probably less stray flux than with the autocollimating arrangements, and almost any degree of magnification of the spectral image can be obtained.

*Methods of Irradiation.* There are two general spectroscopic methods of monochromatic irradiation: (1) the spectrograph method, in which a spectrograph is used to irradiate a series of objects at one time, each with a different dominant wave length selected from the spectrum; and (2) the monochromator method, in which a monochromator is used for the irradiation of a single object at one time with homogeneous monochromatic energy of a single dominant wave length. When the biological material is in the form of small particles, as in the case of viruses, bacteria, or spores, the spectrograph method may be used by spreading the material on a glass plate inserted in the camera. For large objects, such as small animals or plants, a highly magnified spectrum is required (Parker *et al.*, 1946).

The spectrograph method has the advantages that the spectrum can be covered rapidly and that many individual treatments of diverse wave lengths can be made simultaneously on samples from the same population. The method is especially useful where long irradiation periods are required. However, the use of a series of segments of the full spectrum is limited to the single spectrograph, and no double monochromator or predispersion system can be employed before the entrance slit. Thus the background radiation due to scattering may be significant and not readily eliminated.

With the monochromator method it is much easier to isolate a narrow region of known spectral composition, and predispersion systems can be

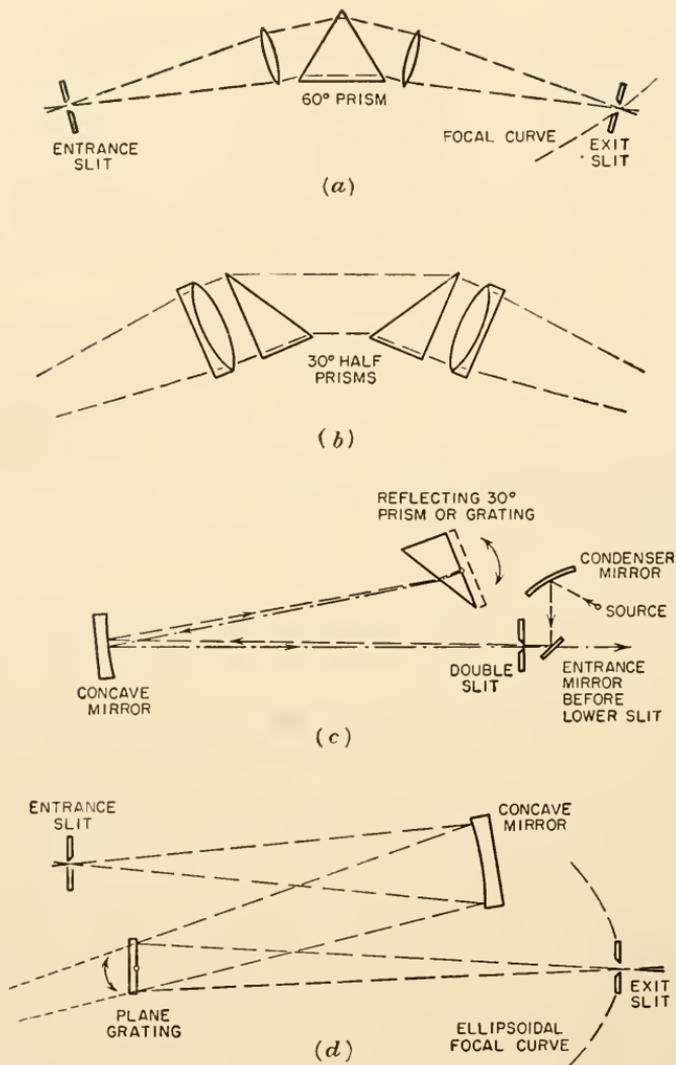


FIG. 3-24. Single-monochromator optical systems frequently used for the ultraviolet and visible. Mounting *a* employs a single prism or several prisms in tandem. Concave spherical or parabolic mirrors may be used in place of lenses to eliminate focusing adjustments for wave length. Mounting *b* is the Thollon biprism arrangement often used in the ultraviolet and visible with crystal quartz prisms. Mounting *c* is the Littrow arrangement used in some commercial spectrophotometers. The dispersing element may be a  $30^\circ$  reflecting prism or a reflection plane grating. The *d* mounting involves a converging beam incident to the dispersing element. Either prisms or reflecting plane gratings may be used. This arrangement is especially useful for producing a magnified spectrum.

employed to eliminate undispersed stray flux. The double monochromator consists of two monochromators in tandem such that the first instrument acts as a predispersion device for the second. This is the most refined method of obtaining a spectrum of great purity, but such instruments are usually available only with prisms and in relatively small sizes and at relatively great cost. With simple predispersion systems consisting of filters or chromatic optical elements, the stray flux of the single monochromator can be reduced by a factor of 10 or more. The interference filter is one of the most effective predispersion elements, since it has a high effective aperture when used in a converging beam before the entrance slit.

### CONTROL OF IRRADIANCE

It is frequently necessary to vary the intensity of the irradiation field with relatively high precision. The three most common methods are (1) by electrically varying the power input to the source, (2) by interposing various types of neutral filters, and (3) by varying the distance of the object from the source.

#### VARYING INPUT

The power input may be varied either by selecting lamp sources of different power ratings, thus varying the intensity in discrete and relatively large steps, or by varying the power input electrically. It will be noted from Table 3-13 for the tungsten-filament incandescent lamp that for any particular class the higher-wattage lamps operate at a higher color temperature and have a higher proportion of energy in the shorter wave lengths than lamps of lower wattage. This may be a significant factor when wide spectral regions are employed.

The intensity of an incandescent lamp can be varied continuously from zero to the maximum rating of the lamp by varying the applied voltage. This method is satisfactory where narrow regions are being isolated and adequate voltage regulation is available. For wide spectral regions the shape of the spectral-energy-distribution curve changes rapidly with increasing voltage, as indicated by increase in color temperature. Some investigators have specified intensity in terms of applied lamp voltage. This yields rather meaningless data, since the irradiance and spectral energy distribution are both complex functions of voltage and the constants vary with the lamp type.

The volt-ampere characteristic of the gaseous discharge lamp has a large negative slope at low values of lamp current, and the required lamp voltage increases rapidly as the current is decreased. For this reason all gaseous discharge lamps become unstable at supply voltages much below the normal rating for the lamp and its ballast. By introducing a variable resistance in series with the lamp, it is usually possible to vary the

intensity by a factor of 5 and still obtain stable operation. However, it must be kept in mind that, if the cathodes of the hot-cathode lamps do not stay sufficiently hot for adequate emission, the resulting high field intensity is apt to damage the cathodes.

The spectral energy distribution of the phosphors of the fluorescent lamp does not change appreciably with change in current or power input. Varying the power by means of a series resistor is a convenient method of controlling the intensity of these lamps over a considerable range.

#### NEUTRAL FILTERS

A wide variety of materials may be employed as spectrally neutral filters for reducing the irradiance by a precisely known factor. These include perforated diaphragms, woven wire cloth or screen wire, evaporated metal films (Banning, 1947), blackened photographic emulsions, dyed films (Eastman Kodak Company, 1951; Withrow and Price, 1953), and neutral gray glasses (Corning Glass Works). Where large areas and distributed sources are involved, perforated metal diaphragms and woven wire cloth are convenient neutral filters. By drilling a large number of small holes in a uniform pattern in thin sheet metal and beveling the edges of the holes to a thin edge, diaphragms can be obtained which have precise transmission factors and are spectrally neutral throughout the ultraviolet, visible, and infrared. If the holes are arranged on a square pattern and are of sufficient number to obtain uniform sampling of the radiant flux from a diffuse source, the following formula may be used in calculating the diameter  $d$  of the holes and the center-to-center spacing  $w$  required for a given transmittance  $T$ :

$$d^2 = 4w^2T/\pi. \quad (3-27)$$

Woven wire cloth may be obtained commercially with specified factors of "open" spaces to total screen area. These factors are approximately equal to the transmittance of a blackened wire cloth. Screens of most metals except aluminum may be blackened with electrodeposited platinum black (Britton, 1951). Copper and high-copper-content alloys, such as brass and bronze, are readily blackened with alkaline oxidizing baths (Raymond) or with proprietary solutions such as Ebanol C.

For very low values of transmittance, screens can be placed in tandem, provided that sufficient distance is interposed between the screens and provided that the wires of the screens are not parallel to each other. A well-collimated beam of parallel flux passing through two screens in tandem can be made to vary in intensity from zero to the maximum allowed by the transmittance of one screen by small variations in position. For this reason, multiple screens should be used with considerable caution.

Neutral filters made of evaporated metal films, photographic emulsions that have been blackened to controlled degrees of density, and gelatin

films containing graphite particles in suspension all decrease in transmittance with decreasing wave length owing to the small size of the absorbing particles that produce Rayleigh scattering. These should be calibrated separately at each wave length and under the conditions of use, since they are never completely neutral over a wide range of wave lengths.

#### INVERSE-SQUARE LAW

Application of the inverse-square law is a useful means for predicting relative irradiances over large values, provided that the source and object to be irradiated are small compared with the distance between the two. Not only must the source be relatively small, but also extraneous reflections from surrounding objects must be eliminated, since they can contribute appreciable additional irradiance. If the surface of the irradiated object is not normal to the direct rays from the source, the Lambert cosine law must be applied.

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#### 4. MEASUREMENT OF RADIANT ENERGY

The precise measurement of radiant flux usually involves three basic instruments: a detector, a measuring device, and a standard radiation

source. The detector converts the incident radiant flux into a signal that is measured quantitatively by an electrical, optical, mechanical, or chemical measuring system. Common examples of detector-measuring instrument systems are the thermopile and galvanometer, the photo-emission cell and vacuum-tube voltmeter, the photographic plate and densitometer, and the eye and visual photometer.

The detector-measuring system is calibrated with a standard source. The primary standard of radiation is the complete, or Planckian, radiator (black body), whose spectral intensity is precisely determined by temperature. In the working laboratory, secondary standards are used which have been calibrated indirectly against a primary standard. A few detectors, such as the pyrhemeters used in solar-radiation research, are absolute instruments whose response can be calculated from the intrinsic properties of the detector system. However, as a class, these detectors are too insensitive for general laboratory use.

Ideally the detector-measuring system is linear in response over the entire range of intensities to be measured, and a plot of the response against flux intensity yields a straight line. The ratio of signal or response to intensity, known as "responsivity" or "sensitivity," is a constant for all values of intensity for a linear detector. Many of the electrical detectors deviate from a linear response by less than 1 per cent over many orders of magnitude of intensity, and the associated electrical measuring instruments can be made even more linear. The photographic plate and the eye are notable examples of nonlinear detectors. For precise measurement they must be used as null detectors for indicating balance between two fields of equal intensity.

#### DETECTORS

The most useful detectors for quantitative measurement produce a change in electromotive force (emf or voltage), current, or resistance. In considering the electrical detectors it is necessary to evaluate them not only in terms of their intrinsic sensitivity and signal/noise ratio, but also in regard to other physical characteristics and the limitations that they impose upon the measuring system. For each type of detector there is a type of electrical system yielding optimum conditions of sensitivity, linearity, and speed.

There are five general classes of detectors for measuring ultraviolet, visible, and infrared radiant energy:

1. Thermal detectors (also known as "radiometers," or temperature or heat detectors). In this class the radiant energy is degraded to heat energy at a blackened receiver. The temperature rise of the receiver is determined by suitable electrical or other means and becomes a measure of the intensity of the incident flux.

2. Photoelectric detector. The photoelectric cell has a photosensitive

surface at which incident quanta eject electrons or produce electronic displacements which appear as a change in electromotive force, current, or resistance.

3. Photographic emulsion. Electrons are displaced in the silver halide crystal lattices. The development process causes reduction to spread to whole crystals and thus amplifies the initial photoeffect. The resulting increase in optical density of the emulsion layer then becomes a measure of the incident energy.

4. Chemical actinometer. A photochemical reaction occurs as a consequence of altered electronic energy levels in the pigment system of the actinometer.

5. Human eye. A photochemical reaction in the retina yields products that ultimately excite the neurons of the optic nerve.

The thermal detectors are relatively nonselective as to spectral sensitivity and are often referred to as "nonselective" detectors. They may be used in any region for which the receiver is "black," and the sensitivity is usually constant, to within a few per cent, from the ultraviolet to at least the middle infrared. They are much less sensitive in the ultraviolet and visible than the other classes of detectors, but at wave lengths longer than about  $5 \mu$  in the infrared the thermal detectors are the only detecting instruments available. Since they can be made with constant and reproducible sensitivity to all spectral regions of photochemical interest, they can be calibrated in absolute units in one region and used with precision in another.

All the other four classes of detectors basically depend upon some form of interaction between the incident photons and the electrons in the photosensitive surface. For each type of system there is a minimum quantum energy representing a minimum frequency or maximum wave length beyond which the quantum energy is insufficient for the transition. Also the absorption of the active surface varies in a complex manner with wave length. For these reasons the spectral sensitivity of the electronic-actuated detectors varies in a complex manner, and the long-wave-length limit or cutoff is usually rather sharp. These four classes of detectors are frequently referred to as selective detectors because of the limited nature of their spectral response.

The selective detectors have a fast response, since electronic displacements occurring in much less than a microsecond are involved. However, the actual speed of measurement is often reduced to milliseconds or longer because of time constants associated with the detector itself or time constants imposed by the measuring system. Some of these detectors can approach theoretical limits of sensitivity in certain spectral regions where the absorption is high, resulting in a quantum efficiency that approaches unity. The sensitivity and spectral response of selective detectors are usually a significant function of previous exposure,

temperature, and other factors, so that for critical work frequent calibration is required.

#### THERMAL DETECTOR

The receiver of the thermal detector consists of a thin highly absorbing, or "black," film whose temperature changes may be determined by (1) the change in electrical resistance of the receiver, as in the bolometer, (2) the electromotive force or voltage produced at a thermojunction in contact with the receiver, as in the thermocouple and thermopile, or (3) the change in gas pressure of a small sealed chamber surrounding the receiver, as in the pneumatic or Golay detector. The general characteristics of thermal detectors have been discussed by several authors (Bell *et al.*, 1946; Ellickson, 1947; Fellgett, 1949; Harrison *et al.*, 1948; Hornig and O'Keefe, 1947; Jones, 1947, 1949a,b). Williams (1948) has reviewed the development of thermal detectors for infrared spectroscopy.

The selection of the most useful temperature detector for any particular application requires evaluation of its performance in regard to the following principal characteristics:

1. Radiant-power sensitivity or responsivity  $S_0$ , the ratio of the output signal to the radiant power incident on the receiver; usually expressed in volts per watt, which is equivalent to microvolts per microwatt.

2. Irradiance sensitivity  $S_H$ , the ratio of the output voltage to the incident irradiance in microvolts per microwatt centimeter<sup>-2</sup>, which is equivalent to  $S_0A$ , where  $A$  is the sensitive area of the receiver; expressed in volts per watt centimeter<sup>-2</sup>.

3. Noise equivalent power  $H_m$ , or noise equivalent intensity NEI, the minimum flux detectable when the detector is operating into an amplifier that has the same time constant as the detector; expressed in watts.

4. Time constant  $\tau$ , the time in seconds required for the receiver to attain 63 per cent ( $1 - 1/e$ ) of its equilibrium temperature following irradiation; expressed in seconds.

5. Resistance  $R$  of the bolometer or thermocouple detector; expressed in ohms.

The radiant-power sensitivity of a thermal detector is approximately inversely proportional to the area of the receiver. Since response is proportional to the rise in temperature induced by the radiant flux and since the dissipation of heat from the receiver is roughly proportional to receiver area, any increase in size of the receiver necessitates a corresponding increase in total flux to produce the same response. Therefore the response of the thermal detector is proportional to the average irradiance. When the detector is used with a monochromator, the flux can be concentrated into a small image; i.e., the irradiance is made as large as possible. The dimensions of the bolometer and thermocouple receivers are made approximately equal to those of the slit image, which are usu-

ally within the range 0.2–0.5 mm in width and 3–10 mm in length. The sensitivity of monochromator detectors is usually specified in volts per watt (or microvolts per microwatt) of continuous flux, with the dimensions of the receiver specified.

In biological research it is often necessary to measure low values of irradiance produced by large sources whose flux cannot be concentrated into a small image. For this application the maximum irradiance sensitivity  $S_0A$  or  $S_H$  is required, and relatively large receivers are used. The power sensitivity  $S_0$  is usually less than with monochromator detectors, but the area  $A$  is large, giving a large value for  $S_0A$ .

The irradiance sensitivity of a thermopile is proportional to the square root of the total receiver area for any specified design, provided that the area is increased without changing the resistance or other physical characteristics. For example, in the case of a thermocouple of a given design, if the area is increased four times by a series-parallel arrangement of four elements which leaves the total resistance unchanged, the voltage sensitivity is only doubled.

The noise equivalent power sets the lower limit to the radiant power that can be detected when an electrical instrument, such as a vacuum-tube amplifier with an equivalent time constant, is coupled to the detector. This is the radiant flux that produces a signal voltage just equal to the noise voltage. However, this is not the lowest radiant power that can be detected if longer periods can be tolerated.

The time constant or speed of thermal detectors is controlled by the thermal mass or heat capacity of the receiver and the rate of heat exchange with the surroundings. The time constant is made small by using thin receivers and by rapidly dissipating the heat. Fast detectors of high sensitivity are achieved by the use of the thinnest receiver materials and by reducing the rate of heat flow from the receiver to a minimum. The receiver loses heat by radiation, conduction, and convection. The radiation loss per unit area is determined by the receiver emissivity and temperature, and little can be done to reduce it. The conduction losses are minimized by the use of fine wires in bolometers and thermocouples. Convection losses can be practically eliminated by evacuation. Evacuation of fast bolometers and thermocouples frequently results in a 10- or 20-fold increase in sensitivity and an appreciable increase in the time constant owing to the lowered rate of heat dissipation.

The time constant is specified by manufacturers in various ways, such as the time to attain some proportion of maximum deflection after the beginning of irradiation, or the percentage of the zero-frequency signal obtained with various frequencies of modulation or chopping of the incident flux. The most fundamental quantity is  $\tau$ , the time required for the signal to rise to 63 per cent ( $1 - 1/e$ ) of its equilibrium value. When galvanometers are used, fast detectors with time constants of less than

100 msec are of little value. The fast detectors are of special merit for rapid recording and where modulation of the incident flux is used to produce an a-c signal that is relatively uninfluenced by stray flux and readily amplified.

The resistance of the detector determines the method of coupling to the measuring instrument to ensure maximum power transfer. In general, the input resistance of the measuring system should approximately match that of the detector within a factor of 2.

The evacuated bolometer or thermocouple detector requires a rigid window, and even those operating in air require a window to eliminate drafts and convection currents. The window material should have negligible selective reflection and absorption in the spectral regions to be used. Although fused quartz and thin glass windows have adequate transparency for many photochemical applications, both materials absorb the infrared beyond about  $5 \mu$ , which amounts to 10–20 per cent of the flux emitted by radiometric standard lamps. This necessitates a large calibration correction for window losses. Calcium fluoride (fluorite) is probably the most satisfactory window material, since it transmits freely from the ultraviolet to beyond  $9 \mu$  in the infrared, and errors due to uncertainty of corrections for selective window absorption are negligible. Calcium fluoride is also relatively nonhygroscopic as compared with other halides, and drying agents are usually not required.

*Bolometer.* The radiation bolometer is, in principle, a resistance thermometer in which the resistance element consists of a thin blackened strip of metal or other conductor with a high temperature coefficient of resistance. If the receiver element is one arm of a balanced Wheatstone bridge, the temperature rise due to absorption of radiant flux can be measured as a change in resistance. The bolometer responsivity can be expressed as a voltage change per watt of incident flux, with a specified bridge current flowing through the receiver. The sensitivity of a bolometer is roughly proportional to the current up to values that produce excessive heating of the receiver element. The sensitivity can be varied over a wide range by varying the current. Vacuum bolometers are frequently operated at temperatures up to  $50^{\circ}\text{C}$ .

When only one bolometer element is used, it is not possible to distinguish readily between changes in intensity of the radiant flux being measured and random fluctuations in scattered flux and ambient temperature. These fluctuations may be balanced out of the bridge response by a compensating element or compensator that is identical in form with the receiver and is made a second arm of the bridge (Fig. 3-25). By careful construction as much as 95 per cent compensation can be attained, and further compensation can be effected by shunting a large variable resistance  $R_s$  across the more sensitive element.

Bolometers designed for use with monochromators have the receiver

and compensator facing in the same direction so that either element can be used as the receiver and both elements receive the same scattered flux. The slit image is then focused on only one element. When measuring the flux from a large source, it is not possible to produce a small image, and the compensator must then be shielded internally or placed behind

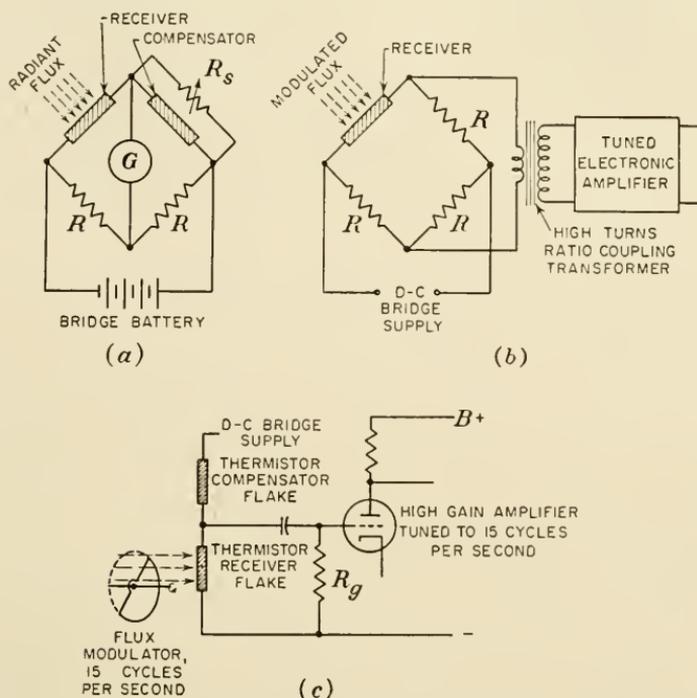


FIG. 3-25. Diagram of the radiation bolometer and coupling circuits for continuous and modulated flux. Arrangement *a* is the classical system employing a galvanometer. All four arms of the bridge have approximately the same resistance.  $R_s$  varies the sensitivity of the compensator arm so that both receiver and compensator have equal sensitivities to stray flux. The arrangement at *b* is suitable for chopped or modulated flux. The low-resistance metal bolometer is coupled to the tuned amplifier through a high-turns-ratio transformer. The high-resistance thermistor bolometer may be coupled directly to the grid circuit of an amplifier through a capacitor, as shown at *c*.

the receiver. These arrangements effectively compensate for fluctuations in ambient temperature but do not compensate for fluctuations in scattered flux.

1. Metal. The receivers of metal bolometers consist of thin strips of metal produced by rolling fine wires, by electrodeposition on a backing plate such as thin sheet copper which can be dissolved away by appropriate reagents (Brockman, 1946), and by vacuum evaporation or sputtering of metal on thin backing films of a plastic (Billings, Barr, and Hyde, 1947). Ribbons produced by rolling wire or by electrodeposition can be produced and handled as thin as  $0.1 \mu$ . By evaporating the metal in a

vacuum on a permanent backing of nitrocellulose or other plastic, receiver elements of less than  $0.01\text{-}\mu$  thickness can be obtained. Platinum with a temperature coefficient of resistance of 0.3 per cent per degree centigrade and nickel with a value of 0.6 per cent per degree centigrade have been used most extensively. The metal bolometers usually have a relatively low resistance in the range 10–100 ohms. The evaporated-nickel bolometer described by Billings, Barr, and Hyde (1947) had an area of  $6\text{ mm}^2$ , a time constant of 4 msec, and a minimum detectable power of  $3.3 \times 10^{-8}\text{ w}$ , with a modulation frequency of 30 cps and an amplifier with a 100-cps band width. The minimum detectable irradiance is about  $0.5\text{ }\mu\text{w cm}^{-2}$  under these conditions. Reduction of the band width to 1 cps or the use of a slow meter can reduce the minimum detectable power by a factor of 10.

2. Thermistor. Thin, blackened semiconductor flakes composed of a mixture of oxides, including those of nickel, manganese, and cobalt, have a negative temperature coefficient of about 4 per cent per degree centigrade and a very high specific resistance. The temperature coefficient of such semiconductor flakes, commonly called "thermistors," is about ten times that of the common metals. Becker and Moore (1946) developed the Sett element, or thermistor bolometer, for infrared use, and Wormser (1953) gave extensive data on its performance. A thermistor bolometer with a receiver 0.2 mm wide, 2.5 mm long ( $0.5\text{ mm}^2$ ), and  $10\text{ }\mu$  thick has a resistance of the order of 3 megohms; therefore it can be coupled directly to the grid circuit of a vacuum-tube amplifier. Since it is not necessary to evacuate these instruments, they are usually mounted directly on quartz or glass or supported in air. The sensitivity and time constant, as given in Table 3-16, vary with the method of mounting. The shortest time constant and lowest sensitivity are obtained with the quartz-backed element, whereas the slowest bolometers with the maximum sensitivity are mounted in air. As with metal bolometers, two elements are mounted in the same housing so that only one element receives the incident flux.

3. Superconductor. Andrews *et al.* (1946) have made use of the very large temperature coefficient of resistance of certain types of conductors near their superconducting range of temperature, where the resistance is approaching zero. The superconducting bolometer (Fuson, 1948) has the highest sensitivity of all thermal detectors, but the inconvenience of operating at the extremely low temperature of about  $15^\circ\text{K}$  required for the niobium nitride conductor eliminates it at the present time as a practical detector for general use. The minimum detectable power is about  $6 \times 10^{-10}\text{ w}$  (Bell *et al.*, 1946).

4. Bolometer circuits. A bolometer detector may be coupled to the measuring device through (1) a d-c Wheatstone bridge and galvanometer, (2) a simple Wheatstone bridge supplied with alternating current, the

unbalance a-c emf being detected with an a-c amplifier, or (3) a two- or four-arm bridge supplied with direct current but coupled to an a-c amplifier that amplifies the a-c component of the signal produced by modulating or chopping the incident flux.

The d-c bolometer bridge and galvanometer operating at high sensitivity always exhibit an uncertain drift of the zero position. This is principally due to fluctuations in thermoelectric emf and resistance caused by minute temperature variations occurring in various parts of the circuit, and to variations in stray radiant flux.

The zero drift due to slow changes in the thermal emf at each junction of the bolometer circuit can be eliminated by supplying the bridge with alternating current and detecting the unbalance with a conventional a-c amplifier that is tuned to the frequency of the bridge power supply (Moon, 1935; Schlesman and Brockman, 1945). The sensitivities obtainable are equivalent to those of the most sensitive galvanometer systems and are limited only by the thermal, or Johnson, noise in the resistance elements of the bridge. However, this system does not eliminate zero drift due to small fluctuations in resistance of the bridge components or fluctuations in stray flux.

Zero instability due to fluctuations in resistance, thermoelectric emf, and stray flux can all be reduced to second-order variations by modulating or chopping the radiant flux at the source and using a fast bolometer in an a-c bridge circuit connected to a tuned amplifier. Modulation frequencies of 5–30 cps are commonly used. It is important to recognize that fluctuations in stray flux are eliminated from the signal only if the source itself is modulated and the stray flux is not. The chopping device, which frequently consists of a rotating sector, should be placed at the source and not at the detector.

The metal bolometer, with its low impedance and voltage sensitivity, requires a high-turns-ratio coupling transformer for impedance matching it to the grid circuit of an a-c vacuum-tube amplifier (Fig. 3-25). Turns ratios of 100–750 have been used with metal bolometers and thermocouples (Robinson, 1952). Thermistor bolometers are almost universally used with chopped or modulated incident flux at frequencies up to 15 cps. The thermistor-bolometer bridge (Fig. 3-25) consists of only two arms instead of the usual four, since only the a-c component is amplified. The bridge voltage is usually between 100 and 400 v dc. The minimum detectable power, as limited by signal/noise ratios, is of the same order of magnitude for both metal and thermistor bolometers when properly coupled to vacuum-tube amplifiers.

*Thermocouple and Thermopile.* The radiation thermocouple consists of a pair of thermojunctions in which one, the hot junction, is in contact with a blackened receiver. The cold junction is connected either to a similar receiver, which may be used as a compensating element for stray

flux, or to a thermal sink consisting of a relatively large mass of metal, which may be the housing. Compensating thermocouples (or thermopiles) are commonly made of two complete couples connected so that the generated voltages oppose one another. In this case the cold junctions are connected to thermal sinks. The thermopile is composed of two or more thermocouples arranged in series or series parallel to increase the receiver area and generated voltage.

When a circuit is composed of two dissimilar metals, a contact potential is present at each of the two junctions. If the two junctions are of the same temperature, the contact potentials are equal and opposite, and no current flows in the circuit. If, however, the junctions are at different temperatures, a current will flow which is proportional to (1) the temperature difference  $\Delta T$  and (2) the algebraic sum of the thermoelectric powers of the two metals  $Q$  and inversely proportional to (3) the resistance of the circuit  $R$ . Thus  $I = KNQ \Delta T/R$ , where  $N$  is the number of pairs of junctions in the circuit ( $N$  is 1 for a thermocouple). The ideal pair of thermoelectric materials has a high thermoelectric power  $Q$  and a low specific resistance  $\sigma$ . Of the many combinations of metals available for radiation thermocouples, copper or manganin with constantan, and bismuth with bismuth-tin alloys probably have been used most extensively. The construction of fast thermocouples and thermopiles with fine wires has been described by many authors (Harris, 1946; Hornig and O'Keefe, 1947; Roess and Dacus, 1945). Certain semiconductors involving alloys of tellurium in combination with bismuth offer the highest thermoelectric

TABLE 3-16. COMPARATIVE PERFORMANCE OF THERMOCOUPLES AND BOLOMETERS

Description of detector	Time constant, msec	Receiver area, $A$ , $\text{mm}^2$	Power sensitivity, $S_0$ , $\text{v w}^{-1}$	Irradiance sensitivity, $S_H = S_0 A$ , $\text{w w}^{-1} \text{mm}^{-2}$	Resistance, $R$ , ohms
Thermocouples and thermopiles					
Eppley.....	90	1.0	0.4	0.4	6
Hornig, Farrand.....	40	0.5	8	4	8
Hornig and O'Keefe.....	35	0.5	6.5	3	5
Hornig and O'Keefe.....	40	4.0	4	16	12
Liston, Perkin-Elmer.....	20	0.4	10	4	20
Reeder, RP3.....	30	0.4	6	2.4	10
Reeder, RUM7.....	65	3.2	4	12.8	5
Reeder, RUM4.....	90	10	2	20	4
Bolometers					
Polaroid, nickel.....	5	4.5	1.5	6.8	64
Thermistor, S-19.....	6	0.6	730	440	$3 \times 10^6$
Thermistor, XB-108.....	140	0.6	3500	1880	$3 \times 10^6$

powers known, but the specific resistance of the semiconductors is so high that a pin type of mounting must be used. The ultimate sensitivity obtainable is not much greater than that for the all-metal couples (Liston, 1947).

Fast thermocouples are obtainable by using extremely fine wires welded or soldered to thin receivers of metal foil and by evaporating alloys onto thin films of supporting materials such as nitrocellulose, as in the construction of evaporated bolometers. Both types of construction have yielded low time constants. Comparison of several typical thermoelectric detectors is given in Table 3-16.

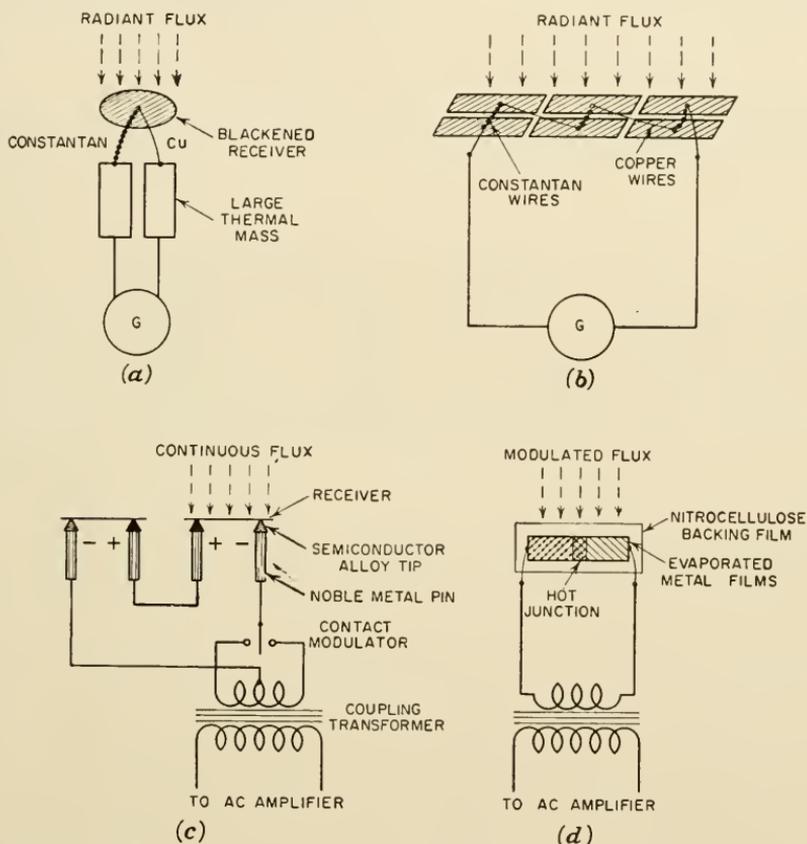


FIG. 3-26. Diagram of various types of thermoelectric detectors and coupling circuits. An uncompensated constantan-copper wire thermocouple is shown at *a*. The linear thermopile of *b* is partially compensated by attaching the cold junctions to blackened receivers. The radiant flux being measured is focused on only one set of receivers. A compensated pin-type thermocouple is shown at *c*. Two couples and receivers are connected in electrical apposition. The output is converted to alternating current by the contact modulator and coupled to a tuned amplifier through a high-turns-ratio transformer. The evaporated film-type thermocouple of *d* has a short time constant and responds to flux modulated at low frequencies.

The principal advantage of the thermocouple or thermopile is that it requires no external source of power and its sensitivity is relatively constant. Evacuation produces essentially the same gain of 10- to 20-fold as with the bolometer and a decrease in speed. The ultimate sensitivities in terms of signal/noise ratios obtainable with thermocouples cover the same range as those for the bolometers. In general, however, the thermocouple is a low-resistance element, and a high-turns-ratio coupling transformer is required for modulated flux. Transformers with turns ratios of 100-750 are available for couples with resistances in the range 10-50 ohms (Robinson, 1952). The thermocouple is coupled directly to the transformer primary, and the secondary is direct- or capacity-coupled to the grid of the first stage, as shown in Fig. 3-26.

*Pneumatic Detector.* The pneumatic detector was first described by Hayes and developed by Golay (1947a,b) into an instrument of great reliability and sensitivity. It is one of the most sensitive and fastest

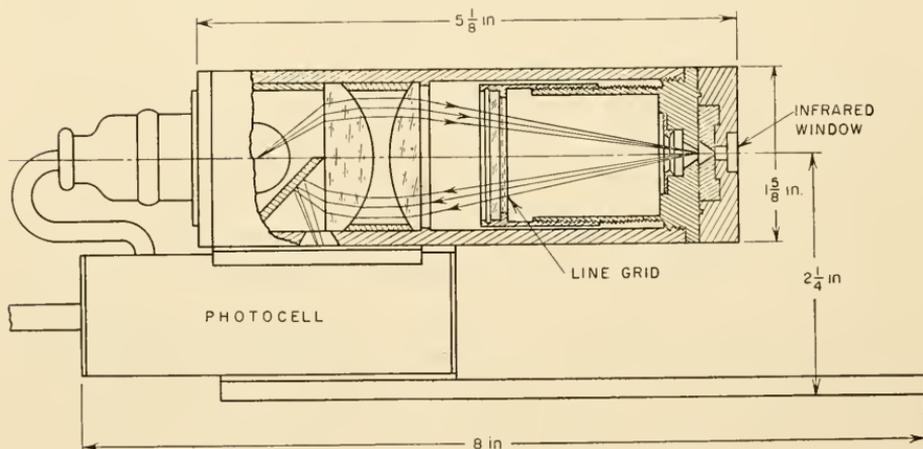


FIG. 3-27. Diagram of the Golay pneumatic detector. (From Golay, 1947b.)

of the thermal detectors. The Golay pneumatic cell consists of a small chamber containing a receiver of a very thin evaporated film of metal in the center, a window at one end, and a very thin flexible mirror at the other. The metal film, which is about 3 mm in diameter, absorbs the radiant flux entering the cell through the window, heats the surrounding gas, and causes pressure changes that are detected optically by the distortion of the flexible mirror, as shown in Fig. 3-27.

The flexible mirror is placed at the focus of an optical system so that it reflects an image of a line grid back onto another portion of the same grid in such a manner that small changes in curvature of the mirror produce large movements in the grid image and large changes in transmitted flux. A photoelectric cell continuously measures the intensity of the flux passing through the line grid. The radiant flux to be measured is modulated

at 10 cps, so that the vibrations of the flexible mirror produce an a-c signal in the photocell circuit. The a-c signal is amplified and rectified for operating a d-c recorder or meter.

When properly adjusted, the Golay detector has a linear response over a wide range of intensities. The noise equivalent power is about  $10^{-9}$  w (Table 3-16). When the detector is operating into a recorder with a full-scale deflection time of 1 sec,  $0.6 \times 10^{-10}$  w or  $10^{-9}$  w  $\text{cm}^{-2}$  can be detected. The time constant of the Golay detector element is less than a millisecond but can be varied over a wide range. The sensitivity is relatively uniform from the ultraviolet through the infrared to the microwave region.

#### PHOTOELECTRIC DETECTORS

The theoretical principles of operation and application of all types of photoelectric cells have been extensively covered by Zworykin and Ramberg (1949). Three types of photoelectric detectors are in general use: the photoemissive, the photoconductive, and the photovoltaic cells. The photoemissive cell consists of a photosensitive cathode from which electrons are ejected as the result of photon absorption. The ejected photoelectrons are collected by an anode. In the photoconductive cell, photon absorption causes the displacement of electrons from semiconductor crystal lattices, leaving "holes" into which other electrons may migrate. The resistance of such a cell is a function of the irradiance. The photovoltaic cell contains a semiconductor film sandwiched between two electrodes and generates an emf when irradiated. It requires no external power supply and can operate a microammeter directly.

The selection of the proper photocell for any research application involves consideration of seven primary characteristics: (1) relative spectral sensitivity, (2) flux sensitivity, (3) dark current and noise, (4) linearity, (5) stability and fatigue, (6) time constant, and (7) electrical characteristics. The relative spectral sensitivity is intrinsically determined by the nature of the photosensitive surface and modified by the transmission characteristics of the window, if one is present. For each type of surface there is a long-wave limit or spectral threshold beyond which the quanta have insufficient energy to produce the necessary electron emission or transition.

For those photocells with a high sensitivity in the visible, the flux sensitivity is usually expressed as the microampere or microvolt per lumen for an incandescent lamp of specified color temperature, which is often 2870°K. The sensitivity of infrared cells is expressed as the microvolt or microampere per microwatt of radiant flux from a specified color temperature, which is usually in the range 300°–600°K. The ultraviolet cells are rated in microwatts at a specified wave length or on the basis of the therapeutic unit of ultraviolet flux, the E-viton.

The power sensitivity multiplied by the sensitive area in suitable units gives the irradiance sensitivity. The power sensitivity in microamperes per lumen multiplied by the area in square feet gives the irradiance sensitivity in microamperes per foot-candle. If it is multiplied by the area in square meters, the units become microamperes per lux.

Every photoelectric cell produces a small but measurable signal when it is receiving no direct radiant flux. This is known as the "dark current" or "dark signal." This current has a random fluctuation with time and establishes the ultimate minimum value of flux which can be measured with d-c instruments. The component of rapid fluctuations in the dark signal becomes the noise that determines the lowest limit of measurable flux when a-c methods are used.

The linearity of the response of photoelectric cells primarily depends upon the cell type. The vacuum photoemissive cell, including the photomultiplier, produces a signal that is proportional to the intensity over many orders of magnitude. The gas photoelectric cell and the photovoltaic cell are reasonably linear in response only with certain circuit arrangements and are usually not employed where extremely linear behavior is essential.

The sensitivity of all photoelectric cells varies with time, previous exposure to radiant flux, and temperature. The slow decrease in sensitivity during irradiation is known as "fatigue" and sets a definite limit to the precision with which intensity ratios can be determined in photometry. The fatigue characteristics must also be taken into consideration whenever photoelectric cells are calibrated against standard thermal detectors or standard sources and later used as calibrated detectors.

The time constant of the photoemissive cells is so small that it seldom becomes a consideration of consequence. Usually the time constant of the input measuring circuit is the limiting factor. The photoconductive and the photovoltaic cells, however, have relatively long time constants which may limit modulation frequencies to certain ranges in the audio-frequency spectrum.

The electrical characteristics of a photocell determine the optimum circuitry required. The principal considerations are the nature of the signal and the internal impedance. The photoemissive cell has a very high impedance, whereas the voltaic cell has a relatively low impedance. The electrical characteristics of the external circuit also determine the linearity of response of the photovoltaic cell.

*Photoemission Cell.* The photoemission cell (Fig. 3-28) is available in three general forms: (1) the vacuum photocell, (2) the gas photocell, and (3) the secondary-emission photomultiplier.

The emission of electrons by photosensitive surfaces is governed by two principles: (1) the number of electrons emitted per unit of time is directly proportional to the incident flux (number of quanta per second),

and (2) the kinetic energy of the released electrons is inversely proportional to the wave length (proportional to the frequency) and independent of the incident flux. These relations arise from the fact that an electron must have an energy in excess of a minimum value  $W$  in order to be ejected. The quantum energy  $h\nu$  must then be equal to or greater than

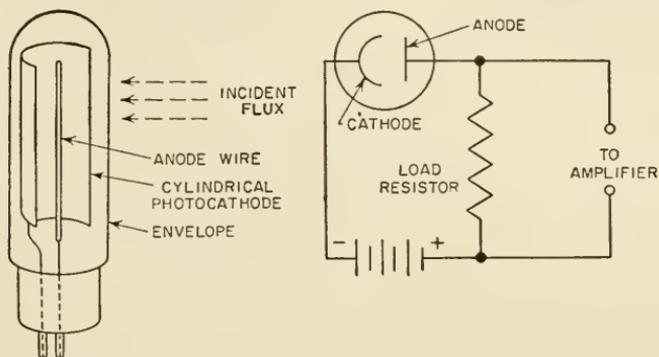


FIG. 3-28. The photoemission cell and the circuit used to couple it to a vacuum-tube amplifier.

$W$ . The excess energy resulting from the interaction between a photon and an electron at a photosurface is converted into kinetic energy, and

$$\frac{1}{2}mv^2 = h\nu - W.$$

At absolute zero  $W$  has a minimum value. At higher temperatures some of the electrons will have acquired additional energy as a result of thermal agitation, and a few will have sufficient energy to leave the surface in the absence of any radiant flux. This very weak emission constitutes the thermionic emission, which becomes a large part of the dark current at room temperature for cells sensitive to the near infrared. The minimum energy requirement is a constant for each type of surface and determines the long-wave-length limit, or photoelectric threshold, since the photon (quantum) energy must be equal to or greater than  $W$ .

The photosensitive surfaces of photoemissive cells are prepared by evaporating various metals onto a sheet-metal surface which serves also as the cathode. The near-infrared, visible, and near-ultraviolet sensitive cells have surfaces that consist of layers of halide and other metals and their oxides. American manufacturers have standardized on a limited number of spectral-response curves which are typical of certain types of photosensitive surfaces. The compositions of representative photosensitive surfaces are given in Table 3-17, and the relative spectral sensitivity in Fig. 3-29. It should be noted that the spectral-response designation S applies only to the spectral-response curve and that compositions other than those given in Table 3-17 may give the same curve.

The S-1 response has two maxima, one in the middle ultraviolet and

the other in the near infrared at  $800\text{ m}\mu$ . It has a high sensitivity to beyond  $1000\text{ m}\mu$ . Because of the high near-infrared response the S-1 surface has the highest thermionic emission and therefore the highest

TABLE 3-17. TYPICAL COMPOSITIONS OF THE PHOTSENSITIVE SURFACES GIVING THE VARIOUS STANDARD SPECTRAL-RESPONSE CURVES

Response Designation	Typical Composition
S-1	Silver-cesium oxide-cesium
S-3	Silver-rubidium oxide-rubidium
S-4	Antimony-cesium
S-5	Antimony-cesium in high-silica glass, as Vycor or quartz
S-6	Sodium in high-silica glass
S-8	Bismuth-cesium

dark current. The S-3 response is high throughout the visible and most nearly approximates the spectral response of the eye. The S-4 and S-5 response are due to the same photosensitive surfaces and differ only in the transmittance of the envelope.

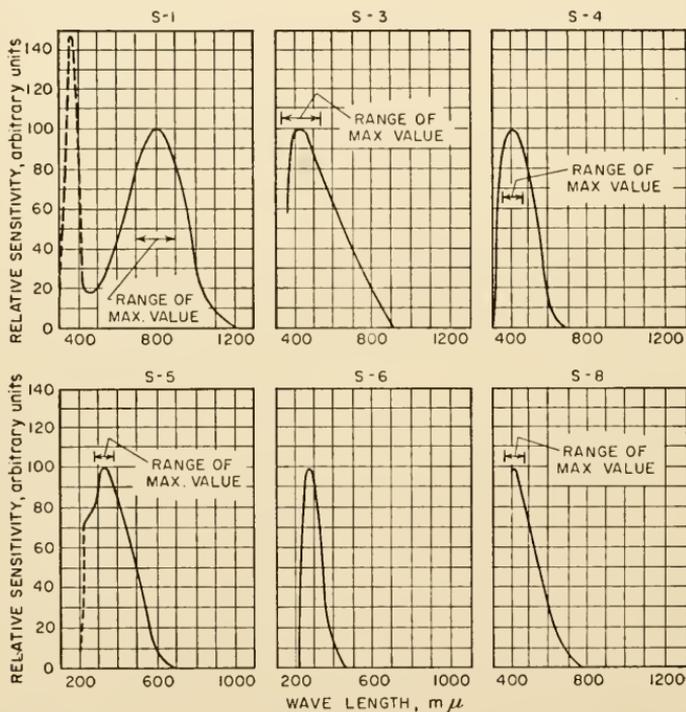


FIG. 3-29. Relative spectral sensitivity of various types of photocathodes. (Courtesy of Radio Corporation of America.)

The vacuum photocell is thoroughly evacuated in order to eliminate collisions between electrons and gas molecules. These collisions produce ions that usually increase the noise level and affect the linearity of the response. The vacuum photocell has an extremely linear response, since

the number of photoelectrons emitted by the photocathode is proportional to the intensity over many orders of magnitude. At constant flux intensity the photocurrent at first rises rapidly with applied voltage and then saturates in the region of 20–30 v (Fig. 3-30). In the region of saturation the current does not appreciably increase with voltage and is therefore relatively insensitive to small changes in applied voltage. The combination of linearity and insensitivity to small voltage changes makes

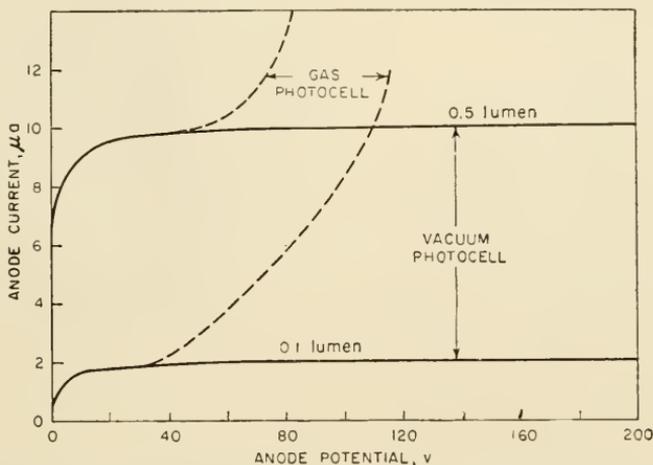


FIG. 3-30. Current-voltage characteristics of vacuum (solid curves) and gas (dashed curves) photoemission cells at two values of incident flux.

the high-vacuum photocell especially useful for precise quantitative measurement.

The gas photoemission cell contains a readily ionizable gas, such as argon, at low pressure. Inelastic collisions between the electrons and gas molecules result in the formation of ions that cause a large increase in the current. The use of an ionizable gas at low pressure was one of the first methods of internally amplifying the photoelectric current. Gas amplification factors of 5 to 15 can be obtained with commercial gas cells, and stable operation still be maintained. The principal disadvantages of the gas photocell are that (1) the response is no longer a linear function of intensity if the voltage is high enough to produce appreciable amplification; (2) the sensitivity is markedly influenced by applied voltage, as shown by the curves of Fig. 3-30; and (3) the gas cell has a relatively high noise level under most conditions of operation. The gas photocell is used principally for nonquantitative applications, such as in the operation of relays where linearity and constancy of behavior are not important.

The photomultiplier tube is a high-vacuum photocell containing an internal current-amplifying system (Engstrom, 1947; Marshall *et al.*

1948; Rodda, 1949). Figure 3-31 presents diagrammatically the electrode arrangement of a typical nine-stage secondary-emission photomultiplier tube, the 931-A, which employs electrostatic-field focusing of the electrons. A photoelectron emitted by the photocathode *O* is accelerated by a potential of 40–100 v to dynode 1, where several secondary electrons are released. The secondary electrons are again accelerated to dynode 2, and each one again releases several new secondary electrons. The process is repeated from dynode to dynode until finally the avalanche

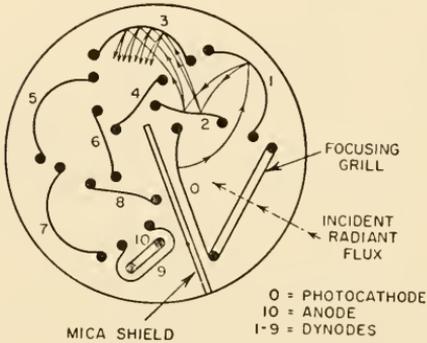


FIG. 3-31. Diagram of the electrode arrangement in a nine-stage electrostatic-focusing photomultiplier tube of 931-A type. The paths of the photo- and secondary electrons are indicated for the first two stages. (From Engstrom, 1947.)

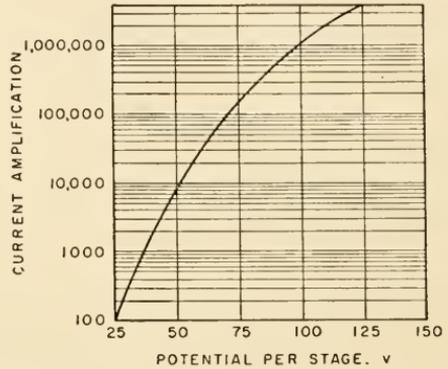


FIG. 3-32. Effect of dynode voltage on the current amplification in a nine-stage 931-A-type photomultiplier tube. (From Engstrom, 1947.)

of electrons is collected by electrode 10, the anode. If the secondary-electron yield is  $R$  per primary electron striking the dynode, the number of dynodes is  $n$ , and the photocurrent is  $i_0$ , then the anode current is  $R^n i_0$ . The secondary-electron yield varies from 1 to 10 depending upon the nature of the dynode surfaces and the accelerating potential between them. For the 931-A multiplier with a potential of 100 v per dynode, the yield  $R$  is over 4, which for nine stages gives an amplification of about a million.

The accelerating potentials may be obtained from a bank of small hearing-aid-type 90-v B batteries or from a voltage divider across a regulated power supply. The power supply must be very well regulated, however, because, unlike that of the vacuum photoelectric cell of two electrodes, the response of the multiplier is extremely sensitive to applied voltage. The current amplification, and thus the over-all sensitivity, is nearly proportional to the logarithm of the applied voltage, as shown in Fig. 3-32. Varying the voltage per stage from 75 to 100 v produces nearly a tenfold increase in sensitivity.

At constant flux intensity and applied voltage, photomultipliers exhibit

fatigue, and the sensitivity decreases with time; the decrease increases rapidly with intensity, and if exposed to very high intensities, the cell may be permanently damaged owing to excessive current on the later dynode stages. Therefore the photomultiplier is seldom used for precise measurements at high flux intensities. It is especially useful at very low intensities.

The photomultiplier has a dark current consisting of three components: ohmic leakage, amplified thermionic emission, and regenerative ionization (Fig. 3-33). Ohmic leakage is due to traces of conducting materials on the stem and insulating elements inside the tube which are unavoidably deposited during manufacture. It is an inherent property of any tube and varies considerably among tubes. Ohmic leakage predominates at low values of applied voltage. At normal voltages per stage of 80–100 v for the 931-A, thermionic emission is predominant and sets the limit to the lowest flux intensity that can be measured. Regenerative ionization occurs only when potentials of 110 v or more are applied to the electrodes. It is caused by electrons ionizing the small trace of residual gas present in the tube. Unless limited, regenerative ionization will cause the complete destruction of the multiplier.

The photomultiplier is extremely linear in its response to intensity (Fig. 3-34). Commercial tubes (Engstrom, 1947) have a maximum deviation of 3 per cent from linearity over a millionfold range of  $10^{-10}$  to  $10^{-4}$  lumen, and this linear behavior appears to extend down to  $10^{-13}$  lumen, where single-electron counting must be used.

Only those photosurfaces which are insensitive to the longer wave lengths in the red, as the S-4, S-5, and S-8 responses, are used in photomultipliers in order to limit the dark current. The red-sensitive silver-cesium oxide-cesium (S-1) surface is not used in commercial tubes, partly because of its high sensitivity in the near infrared and partly because of technical difficulties of manufacture. The dark current due to thermionic emission from an S-1 surface would be excessive at room temperatures.

The application of photomultiplier tubes for the measurement of low flux intensities has been discussed by Engstrom (1947) and for scintil-

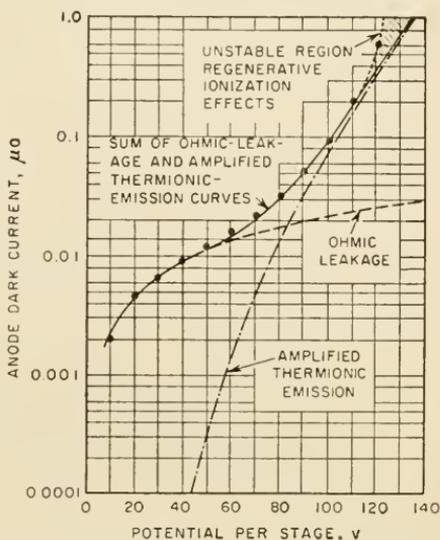


FIG. 3-33. Effect of dynode voltage on the dark current in a 931-A-type photomultiplier tube. (From Engstrom, 1947.)

lation counters by Marshall *et al.* (1948). Selected and paired tubes are stable enough to be used for the detection of small changes in intensity of 1 part in 10,000 (Oldenberg and Broida, 1950). At low intensities fatigue is negligible (Engstrom, 1947). Since the photocurrent of the photomultiplier is internally amplified  $10^4$  to  $10^7$  times, thermal-resistor noise and most of the leakage current present with vacuum photocells are eliminated. The equivalent noise level of the photomultiplier referred

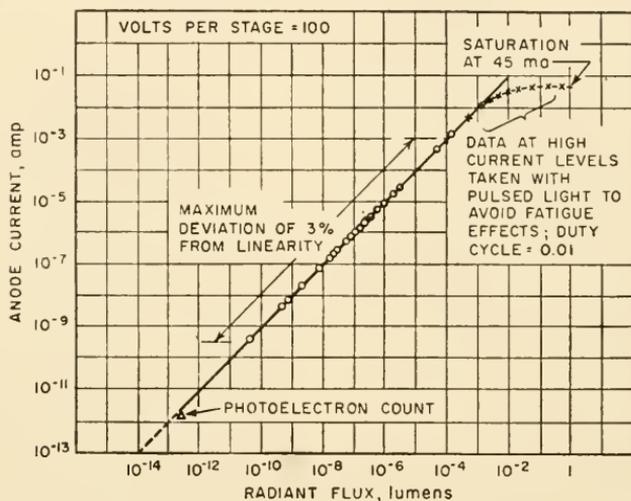


FIG. 3-34. Linearity characteristics of photomultiplier tubes. (From Engstrom, 1947.)

to the photocathode is potentially much less than that of other photocells. The dark current decreases rapidly with temperature, but there is very little decrease in sensitivity (*ibid.*). Reducing the temperature from  $20^{\circ}$  to  $-79^{\circ}\text{C}$  with solid carbon dioxide results in a 20-fold decrease in noise; at the temperature of liquid nitrogen,  $-190^{\circ}\text{C}$ , the noise is reduced about 100-fold.

*Photoconductive Cells.* The electrical conductivity of certain materials, for example, selenium, increases when they are irradiated. A photoconductive cell is obtained when these materials are deposited on an insulator in thin films and electrodes are attached to two opposite edges. The phenomenon of photoconductivity is exhibited by the class of poor conductors known as "semiconductors," which electrically occupy an intermediate position between good insulators, such as quartz and polystyrene, and good conductors, such as the metals. The semiconductors are the active materials in the dry-plate electrical rectifiers and transistors. Those which have been used in photoconductive cells are selenium, germanium, thallium sulfide (thalafide cells), lead sulfide, lead selenide, and lead telluride. Lead sulfide and lead telluride (Moss, 1950; Simpson and Sutherland, 1952; Zworykin and Ramberg, 1949) are of special interest because they extend the range of photoelectric-cell response from the

1200- $m\mu$  limit of the S-1 photoemission cell to 3000  $m\mu$  for the uncooled lead sulfide cell and 5500  $m\mu$  for the liquid-air-cooled lead telluride cell. Up to their respective limits these cells have a much higher sensitivity, as limited by internal noise, than the best of the thermal detectors and are much faster. A lead sulfide film, 0.1–1  $\mu$  in thickness, is deposited either by evaporation *in vacuo* on the inner surface of a glass envelope or by chemical precipitation onto a glass plate. The chemically precipitated cells (Ektron) do not require evacuation and may be used with the film exposed to the air or partially protected by a film of transparent

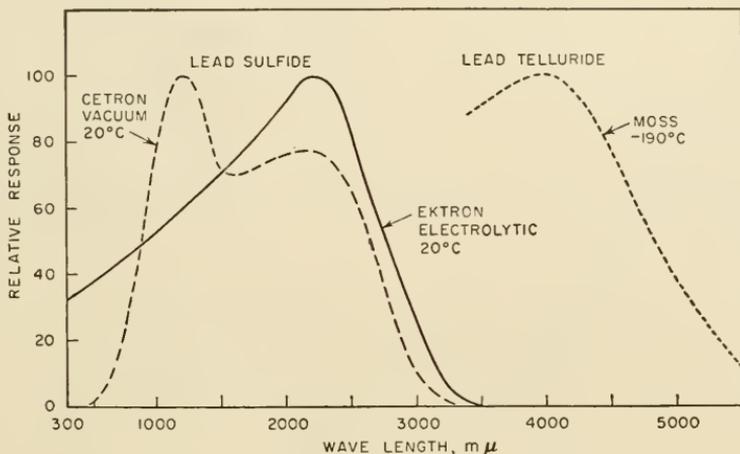


FIG. 3-35. Spectral sensitivity of the lead sulfide and lead telluride photoconductive cells.

plastic. The spectral characteristics vary considerably with the method of preparation; typical curves are given in Fig. 3-35.

The dark resistance for square films is usually in the range from 0.1 to several megohms. The cells are used in a two-arm bridge similar to that given for the thermistor bolometer in Fig. 3-25. The time constant is of the order of 100  $\mu\text{sec}$ , so that flux-modulation frequencies of 60–1000 cps can be used conveniently. Photoconductive cells are seldom used with unmodulated flux because of slow fluctuations in resistance due to temperature and fatigue. It is much more difficult to compensate for these changes by the use of a pair of matched cells than with bolometer elements.

The lead telluride cell has such a long wave-length limit that it is necessary to operate it at the liquid-air temperature of  $-190^{\circ}\text{C}$  to reduce thermal-radiation noise. However, cooling creates difficult practical problems of moisture precipitation on cold windows and the restricting of the thermal flux that enters the window.

**Photovoltaic Cell.** The photovoltaic cell has a low internal resistance and generates sufficient voltage to operate microammeters directly. It

requires no external source of power, as do the photoemission and photoconductive cells. The first photovoltaic cells were of the wet type and consisted of two electrodes immersed in an electrolyte. These cells have been displaced by the more stable and convenient dry, or barrier-layer, cell (Zworykin and Ramberg, 1949),

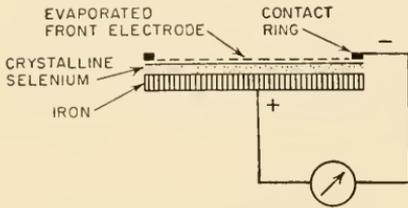


FIG. 3-36. Diagram of the photovoltaic selenium barrier-layer cell.

which consists of a semiconductor layer between a metal backing plate as one electrode and a thin transparent film of metal as the other electrode (Fig. 3-36). The electromotive force is generated at a barrier layer that is in the region of the interface between the semiconductor and one electrode. The most efficient cells

are of the front-wall type, in which the barrier layer is between the thin transparent film on the front surface of the cell and the semiconductor.

Most of the manufactured cells consist of selenium deposited as a thin

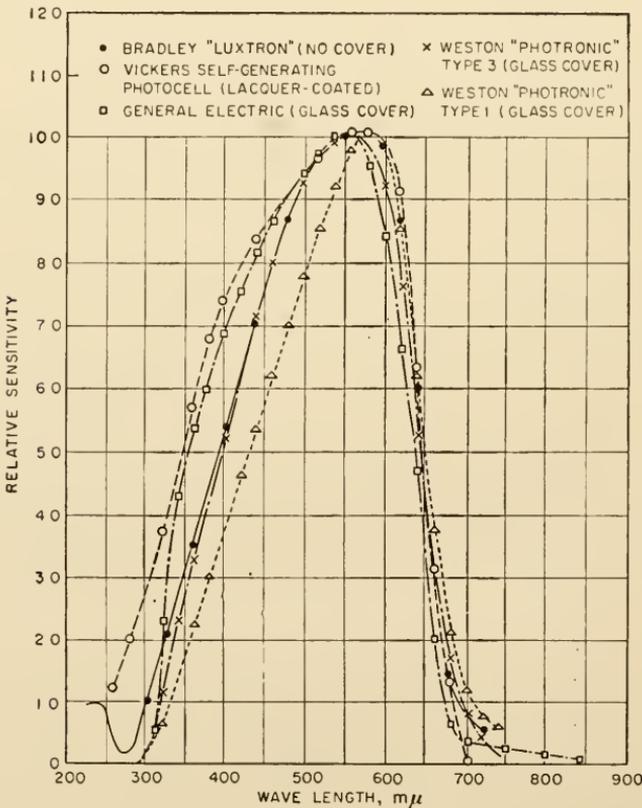


FIG. 3-37. Spectral responses of various selenium barrier-layer cells. (From Zworykin and Ramberg, 1949.)

layer on an iron plate (see Fig. 3-36). The layer is then annealed to return it to its crystalline state. A semitransparent layer of gold or other refractory metal is evaporated, sputtered, or sprayed onto the selenium. Connection is usually made to the top film electrode by a metal ring.

The spectral-sensitivity curve of the selenium cell (Fig. 3-37) is similar to the day or photopic response of the human eye. With a green glass filter, the over-all response can be made to match very closely the luminosity curve of the International Committee on Illumination for the Standard Observer. The Viscon Weston Photronic cell is an example of a filter-corrected photometer detector (Weston Electrical Instrument Corporation; Zworykin and Ramberg, 1949). The filter reduces the over-all sensitivity by about 40 per cent. Filter-corrected sele-

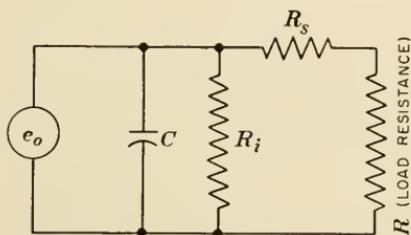


FIG. 3-38. Equivalent circuit of the photovoltaic barrier-layer cell.

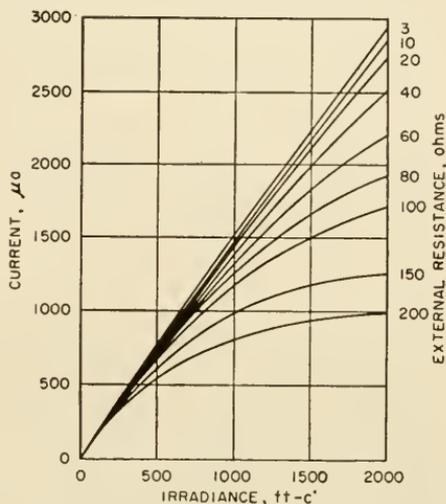


FIG. 3-39. Irradiance-current response curves for the Weston Photronic cell at various values of load resistance. (From Weston Electrical Instrument Corporation.)

mium cells are extensively used in such portable photometric instruments as light meters calibrated directly in the foot-candle or lux. These instruments require periodic calibration, because the best cells slowly deteriorate with time and especially upon exposure to intense sunlight.

The equivalent circuit of a barrier-layer cell is given in Fig. 3-38. It is equivalent to an emf  $e_0$  shunted by a capacity  $C$  and a variable resistor  $R_i$  which are in series with a second resistor  $R_s$ . The shunting resistor  $R_i$  is the resistance of the semiconductor film which decreases with the intensity of the radiant flux. Consequently the external open-circuit voltage is not a linear function of intensity, and the voltage increases less rapidly than the irradiance (Fig. 3-39). It will be noted from the nature of the equivalent internal circuit that, as the load resistance, which may be a microammeter, decreases, the effect of  $R_i$  becomes correspondingly less. At a very low resistance of a few ohms, the response is quite linear, since the shunting effect of  $R_i$  is negligible. Unfortunately this is a condition of inefficient power transfer, because the load resistance is low compared

with the cell internal resistance. Various methods have been proposed for correcting for the variations in the internal shunt resistance (Rittner, 1947).

Whereas the internal emf is generated almost instantaneously, the cell has an appreciable time constant because of the large shunting capacity presented by the small separation of the electrodes. The time constant decreases as the load resistance is made smaller. For a typical cell several centimeters in diameter, the response falls off rapidly above about 1000 cps with a 50-ohm load resistance.

The selenium cell has a cosine error that is especially serious when used to measure the flux from a distributed source, such as the lighted walls of a room or an overcast sky. The response should be proportional to the cosine of the angle of incidence, but it is less than this for the selenium cell. Various methods have been developed for correcting this error by the use of specially shaped diffusing plates of opal glass or plastic (Pleijel and Longmore, 1952).

#### SENSITIVITY LIMITS OF DETECTORS

The measurement of medium to high irradiances above  $10 \mu\text{W cm}^{-2}$  with thermal detectors or 1 ft-c with a selective detector presents few problems. Relatively simple equipment consisting of a thermocouple and lamp and scale, portable galvanometer or selenium cell, and microammeter can be used. It is at the very low levels of intensity that complex electrical instrumentation is required, and consideration must be given to the factors that inherently limit the lowest levels of radiant flux that can be detected. The ultimate limit of sensitivity of a radiation detector is attained when the random fluctuations in the signal, commonly referred to as "noise," as produced by the detector and the associated amplifier, are equal to the signal. The ability to detect a given radiant flux is measured in terms of the signal/noise ratio. The minimum flux that can be detected is generally considered as that which gives a signal/noise ratio of 1. In the thermal detector the noise arises from random fluctuations in the radiation field, temperature of the receiver, and emf or Johnson noise in the resistance elements of the detector. A detector that is in thermal equilibrium with its surroundings is continually absorbing and reradiating energy. The magnitude of the exchange is proportional to the fourth power of the absolute temperature. Since the receiver is an efficient absorber of all radiant energy from the ultraviolet into the far infrared, there is an appreciable component of exchange at  $300^\circ\text{K}$ , where the  $\lambda_m$  is about  $10 \mu$  for a Planckian radiator. The various factors that limit the ultimate sensitivity of radiation detectors have been treated theoretically by several authors (Fellgett, 1949; Golay, 1947a; Hornig and O'Keefe, 1947; Jones, 1947).

Bolometers and thermocouples are electrical resistors that produce a

noise voltage as a result of thermal agitation of the electrons. This noise voltage was experimentally evaluated by Johnson (1928) and theoretically treated by Nyquist (1928) and others (Moullin, 1938). The magnitude of the Johnson noise is given by the formula

$$E_N = (4kTR \Delta f)^{1/2}, \quad (3-28)$$

where  $k$  is the Boltzmann constant,  $T$  the absolute temperature,  $R$  the resistance of the circuit element, and  $\Delta f$  the frequency limits or band pass of the measuring system. The formula reduces to

$$E_N = 7.4 \times 10^{-6}(TR \Delta f)^{1/2},$$

or, for a room temperature of 300°K, to

$$E_N = 1.3 \times 10^{-4}(R \Delta f)^{1/2}, \quad (3-29)$$

where  $R$  is in ohms and  $\Delta f$  in cycles per second. For a 40-ohm thermocouple and an amplifier that passes all frequencies between 1 and 11 cps,  $\Delta f = 10$ , and the Johnson noise is  $E_N = 0.026 \mu\text{v}$ . For a 10-megohm photocell load resistor, it is  $1.3 \mu\text{v}$ .

In the ideal thermal detector, temperature fluctuations in the receiving element determine the ultimate limit of sensitivity. However, in practical detectors there is relatively weak coupling between the radiation field and the electrical signal; i.e., only a small proportion of the radiant power absorbed appears in the signal. As a result of this weak coupling, the sensitivity of practical thermocouples and bolometers is always limited by Johnson noise. The magnitude of this noise signal can be calculated from the resistance of the detector. From the noise voltage  $E_N$  and the sensitivity in volts per watt  $S_0$ , one can calculate the minimum detectable power in watts of radiant flux. If the thermocouple has a sensitivity of  $6.5 \text{ v w}^{-1}$ , the minimum detectable power would be  $4 \times 10^{-9} \text{ w}$ . If a slow meter with a period of about 10 sec is used in the output of the amplifier, the effective band pass for the system is reduced by about 100, and the minimum detectable power is reduced by a factor of 10.

The selective detector, such as the photomultiplier, is inherently much more sensitive in the spectral region of its useful range than the thermal detector, because most of the thermal radiation is outside the region of spectral sensitivity, and the coupling between the radiation field and the electrical signal is much stronger. Maximum coupling is attained when the quantum efficiency is 1 and individual electrons can be counted.

The cooling of both thermal and selective detectors to dry-ice or liquid-air temperatures greatly reduces the magnitude of the stray radiation field, and, in case of thermal detectors, greatly decreases the thermal-resistance noise, as predicted by the Nyquist formula, Eq. (3-29).

## MEASURING INSTRUMENTS

The classical measuring instrument is the galvanometer, and it is still one of the most sensitive and useful. Cartwright (1940a,b) has discussed the theoretical limits of resolution of galvanometers and the various means of attainment. The principal limitation of the galvanometer is the delicate nature of its mechanical system and its sensitivity to vibration. The development of technics for converting d-c signals into a-c signals has made it possible to attain theoretical limits of sensitivity with instruments which are rugged and simple to use and which yield precision superior to that of the galvanometer.

The d-c amplifier is useful for the measurement of the voltage output of high-impedance detectors, such as the vacuum photocell and the photomultiplier. However, it is not possible to separate the small voltage fluctuations of the heater and polarizing voltages of the amplifier from the d-c signal. Therefore the d-c amplifier is seldom reliable for detecting voltage below 1 mv, and it is less suitable than the a-c amplifiers for driving recorders because of the slow but constant drift in the output voltage.

In the a-c amplifier, slow fluctuations in the power-supply voltages are readily separated from the a-c signals that are to be amplified. As a result, the amplified a-c signal is relatively free of drift. The output is usually rectified and converted to direct current for the operation of d-c meters and recorders. If the amplifier has a large amount of inverse feedback, its amplification can be made very independent of supply-voltage fluctuations and tube characteristics, and the output made a linear function of the amplified signal.

Numerous methods have been developed for producing a-c signals. The ultimate objective is to make the over-all system as nearly independent as possible of fluctuations in all extraneous factors associated with the measurement, such as variations in stray flux, temperature, and power-supply voltages. One of the earlier steps taken to eliminate fluctuating factors was to apply alternating current to the bolometer bridge (Moon, 1935). Later advances came with the development of fast detectors which made possible the modulation or chopping of the radiant flux at the source so that the detector would receive a d-c component from the stray flux and an a-c component from the source. Only the a-c component is amplified, and slow random changes in the stray flux do not appear in the output. This system is commonly used in commercial recording infrared spectrophotometers.

When the radiant flux is modulated at constant frequency, a mechanical chopping device is required unless the source is modulated electrically from an a-c supply. Consequently it is often more convenient to convert the d-c signal of the detector into alternating current for amplification. The alternating current can then be rectified for operating d-c

instruments. Of the several systems that have been developed for converting direct to alternating current, the two most commonly used are contact modulation and capacity modulation. Contact modulation involves either a magnetically driven vibrator or a motor-driven switching device for chopping the d-c signal of the detector and converting it into an alternating current. The contact-modulation system is especially suitable for low-impedance detectors, such as thermocouples and bolometers. A motor-driven system employing two sets of gold contacts, one for converting direct to alternating current and the other for

TABLE 3-18. COMPARISON OF CHARACTERISTICS OF SENSITIVE ELECTRICAL MEASURING INSTRUMENTS

Description of instrument	Modulation frequency, cps	Period, sec	Internal impedance, ohms	Voltage sensitivity, $\mu\text{v}$	Current sensitivity, nanoamp ( $10^{-9}$ amp)
Galvanometers					
Leeds and Northrup					
Type HS, moving coil No. 2285-a	...	7.5	15	0.1	7
Type HS, moving coil No. 2285-g	...	7	500	0.15	0.3
Coblentz, moving magnet	...	8	10	0.004	0.4
Kipp and Zonen, portable "light spot," box type	...	3.5	450	0.3	0.5
Contact-modulated "breaker" amplifier, Liston-Becker	8	1	20	0.0006	0.03
Vibrating-capacitor electrometer, Brown	60	4	$10^{11}$	50	$0.5 \times 10^{-6}$
Vibrator amplifier, Leeds and Northrup					
Microvoltmeter	60	1	$10^7$	0.25	
Microammeter	60	1	1000	...	0.005
Vacuum-tube d-c amplifier, electrometer type	...	1	$10^{11}$	1000	$10^{-5}$

synchronous rectification, has been described by Liston *et al.* (1946). Commercial instruments have such a low inherent noise input from the amplifier and contacts that the limiting measurable voltage is determined by Johnson noise in the resistance of the detector.

The capacitor system of modulation in the form of the vibrating-reed electrometer has been described by Palevsky *et al.* (1947) and Reese (1952) for the measurement of ionization currents. It is a high-impedance device and ideally suited to the measurement of the output of the vacuum photoelectric cell and the photomultiplier. Commercial recording instru-

ments of this type are capable of attaining almost theoretical sensitivities, as determined by Johnson-noise limits. Vibrator types of contact modulators with inverse feedback can be designed with input impedances high enough for use with photoelectric cells. Their ultimate sensitivity, however, cannot approach that of the vibrating-capacitor type of electrometer.

Kalmus and Striker (1948) have described a carrier-current system based upon the modulation of the photoelectric current in photoemission cells with an a-c magnetic field. This system offers many of the advantages inherent in the radiant-flux-chopping systems.

Data on the performance of several types of electrical measuring instruments are compiled and compared in Table 3-18. For the electronic instruments the period given is that for the output meter or recorder. The sensitivities are based upon noise limitations for the Liston-Becker amplifier and 0.5 per cent of full scale for the others.

#### STANDARDS OF RADIANT ENERGY

The calibration of sources and radiation instruments is based upon secondary standards of radiant flux, wave length, absorption, and reflection. The standards of radiant flux are incandescent lamps calibrated in (1) absolute units of radiant intensity, (2) color temperature, and (3) luminous intensity. Wave-length standards are gaseous discharge lamps containing elements with distinctive and easily recognized line spectra, and standard glass filters of the rare-earth elements whose absorption bands are intense and narrow. The absorption standards are special colored glasses and inorganic solutions whose transmissions have been precisely determined by the U.S. National Bureau of Standards. The reflection standards usually consist of magnesium oxide deposited on metal surfaces, or solid blocks of pure magnesium carbonate.

#### STANDARD LAMPS

The primary standard of radiant flux, luminous flux, and color temperature is the complete, or Planckian, radiator, whose radiation characteristics are a unique function of temperature. The Planckian standard (black-body standard) is usually a furnace containing a ceramic tube, the temperature of which is controlled by a pool of freezing noble metal (Forsythe, 1937; de Groot, 1948). The furnace is so completely insulated that the whole interior is maintained precisely at the temperature of the freezing metal. The melting points of the noble metals give four reference temperatures: 1336°K (gold), 1825°K (palladium), 2042°K (platinum), and 2716°K (iridium). Tungsten, with a melting point of 3653°K, would make an ideal standard, but ceramic materials capable of withstanding this temperature are not available (Stimson, 1949). The primary standards are maintained by only a few of the leading stand-

ardizing laboratories, such as the U.S. National Bureau of Standards, and are used only for the calibration of secondary-standard lamps (Coblentz and Stair, 1933).

Secondary standards, in the form of incandescent lamps that have been carefully selected, aged, and standardized against a primary standard, can be obtained with a certificate containing the precise operating conditions under which the lamp was standardized (*ibid.*; National Bureau of Standards, 1949; Teele, 1953). It is necessary that these conditions be reproduced accurately because of the critical manner in which the radiant flux emitted by incandescent lamps is dependent upon voltage, current, and ambient temperature. The most important precaution to be observed is to avoid subjecting the lamp to mechanical shock and overvoltage. With careful use a standard lamp should serve the requirements of the average research laboratory for many years without restandardization.

Since the incandescent lamp is a pure resistance at power frequencies, it can be operated on either alternating or direct current with sufficient precision for a secondary standard. The only limiting factor is the availability of meters that are sufficiently accurate for a-c measurements. The less expensive moving iron vane, rectifier, and other types of a-c meter cannot be calibrated directly with a d-c potentiometer. For precise measurements of alternating current, power meters capable of accurate reading on both alternating and direct current must be used. The two common types are the dynamometer and the thermocouple meters, but they both have nonlinear scales and are relatively expensive. In addition, the thermocouple meters are easily damaged by overload. It is often more practical to operate the standard lamp on batteries or a regulated rectifier-type d-c power supply (Fig. 3-40), so that d-c meters can be used.

For any incandescent lamp the current and voltage are dependent variables. Therefore the lamp can be adjusted to the calibrated working conditions with either an ammeter or a voltmeter. In general, the current measurement is preferable because, as the lamp ages, evaporation from the filament causes its diameter to decrease. At constant current, the current density of the filament increases and partially compensates for this loss. The voltage measurement may be used to indicate whether the lamp characteristics have changed significantly.

Although the U.S. National Bureau of Standards recommends operating the standard lamps in a room with a curtain containing a suitable aperture between the lamp and the detector, it is usually more convenient in a small laboratory to operate the lamp in a housing such as that given in detail in Fig. 3-40.

The housing is a plywood box with a hinged cover and two sets of controls. One is for the d-c power supply for the radiometric standard lamp, as already described, and the other is for the 500-w standard of

luminous intensity or color temperature. The box is smaller than the enclosure recommended by the U.S. National Bureau of Standards (Coblentz and Stair, 1933), but the error introduced is small. It is painted flat black on the interior and has suitable openings for ventilation. In the front is a 10- by 15-cm aperture with a sliding shutter

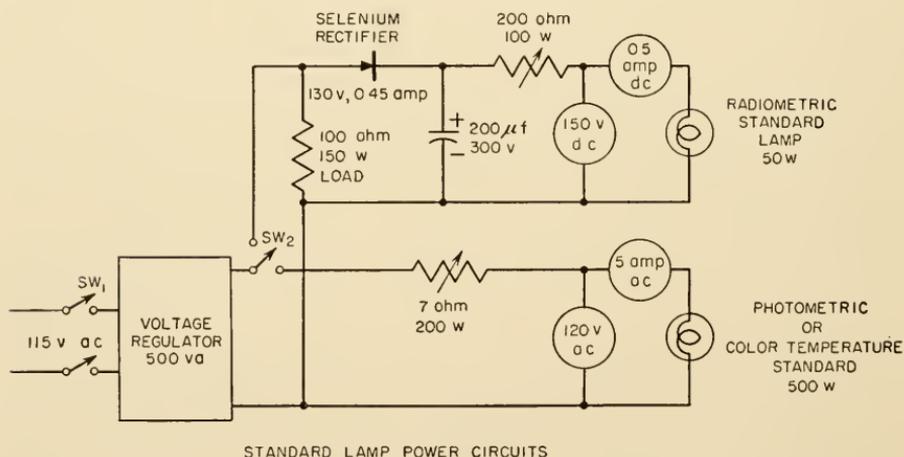
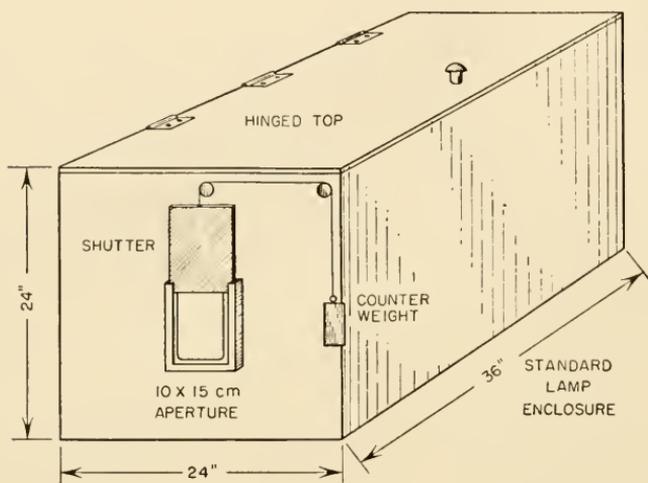


FIG. 3-40. Diagram of a housing for standard lamps. Suitable voltage-regulated circuits for powering the lamps are shown.

that can be opened and closed without bringing the operator's hand into line with the detector. The detector to be calibrated is mounted in front of the cabinet at a distance of 2 m from the standard lamp.

*Radiant Intensity.* The secondary standard of radiant intensity, as routinely issued by the U.S. National Bureau of Standards (*ibid.*) is a nominal 50-w 115-v carbon-filament vacuum lamp that has been cali-

brated against a Planckian radiator in the vicinity of 1300°K, using the Stefan-Boltzmann law for deriving total radiant intensity. With each lamp is a certificate giving three values of current which will produce corresponding values of irradiance in microwatts per square centimeter at 2-m distance from the front face of the lamp bulb. The current and voltage values range from 0.3 to 0.4 amp and from 75 to 110 v. These lamps are especially useful for calibrating thermal detectors such as thermocouples, bolometers, and pneumatic detectors. In Fig. 3-40 is given a simple rectifier circuit for supplying direct current to a 50-w standard lamp.

In calibrating thermal detectors, several precautions must be observed. If the detector has a window of glass, quartz, or fluorite, a correction factor must be applied for window absorption. These correction factors are given in the data accompanying the lamp. The detector should be placed in the room with the calibration equipment for a sufficiently long time to ensure complete thermal equilibration. If there is thermal disequilibrium, an abnormally high drift will be observed, and frequently, when the lamp is turned off, the instrument will not come back to zero. If the zero reading is negative when the lamp is off, it implies that the detector is "seeing" objects colder than itself and is therefore radiating heat to its surroundings. If the zero deflection is positive, it is likely that the surrounding objects are at a higher temperature than the detector. Warm objects, such as power supplies and the body of the operator, must be kept out of the range of the detector.

For the calibration of detectors in the vicinity of 300  $m\mu$ , the quartz mercury arc can be used as a standard. When operated under properly controlled conditions, the high-pressure mercury arc is a reproducible source that depreciates at a predictable rate (Coblentz and Stair, 1936). The mercury arc is preferable to the incandescent lamp as a standard of ultraviolet radiant flux because of the ease with which the ultraviolet lines may be isolated and the relatively high intensity in the ultraviolet as compared with the red and infrared.

*Luminous Intensity.* In order to obtain sufficient luminous intensity for the calibration of foot-candle or lux meters, it is necessary to use a lamp of high wattage and relatively high filament temperature. Because high filament temperature is incompatible with stable characteristics, a compromise has been effected in the form of a 500-w gas-filled projection type of lamp operating at about 300 w (Teele, 1955). These lamps have a luminous intensity in the vicinity of 500 candles.

In the calibration of lux or foot-candle meters, it is necessary to measure the distance from the filament to the photocell (or test plate for visual instruments) and to calculate the illuminance by the formula

$$E = I/d^2, \quad (3-30)$$

where  $I$  = luminous intensity of the standard lamp, candles,  
 $E$  = illuminance, foot-candles (lumens per square foot) or lux  
(lumens per square meter), and  
 $d$  = distance from filament to detector, feet for foot-candle or  
meters for lux.

Since the inverse-square law is employed, the distance  $d$  must always be at least five times the maximum dimension of the filament or the photocell, depending upon which is the larger, in order to keep within the 3 per cent accuracy of most direct-reading photocell-type meters (see Sect. 3 on Inverse-square Law). The general subject of photometric measurements has been treated by Barrows (1951).

*Color Temperature.* A lamp of known color temperature is especially useful as a standard of spectral energy distribution for determining the spectral transmittance or efficiency of monochromators. The various methods of measuring color temperature have been briefly reviewed by Harding (1950). The highest-temperature Planckian-radiator source available for direct color matching is that at the temperature of melting iridium at 2716°K. This color temperature is too low for a standard of spectral energy distribution in the near ultraviolet, and therefore, by means of a blue filter, the U.S. National Bureau of Standards (Judd, 1950; Teele, 1955) extrapolates to a color temperature of  $2854^\circ \pm 8^\circ\text{K}$  ( $C_2 = 14,380$ ) for the calibration of standard lamps to meet the specifications of the International Committee on Illumination for Standard Illuminant A. The color-temperature standard as issued by the Bureau is a 500-w tungsten gas-filled projection lamp designed for 120 v and similar to the photometric standard but operated in the vicinity of 90 v and 400 w. Since this source produces a chromaticity match with that of a Planckian radiator at 2854°K, the spectral-energy-distribution curve within the visible spectrum closely approximates that of a complete radiator operating at that temperature. The spectral-energy-distribution curve can be extrapolated into the near ultraviolet and visible by obtaining the product at each wave length of (1) the relative energy of a complete radiator at the true temperature of the filament, and (2) the spectral emissivity of tungsten for the same temperature, as discussed in Sect. 2 under Thermal Sources. The true temperature or average filament temperature of the 2854°K color-temperature standard lamp is about 2800°K ( $T_f$ ). This temperature is obtained by empirically trying various values of filament temperature and plotting the product of the relative energies for a complete radiator  $J_\lambda$  and the spectral emissivities for tungsten  $\epsilon_\lambda$ . That temperature is selected for the filament temperature which gives the closest fit to a complete radiator at 2854°K within the limits of the visible spectrum. Calculations of this type have been made by Stair (1951). Data for the 2854°K color-temperature standard and the spectral emissivity of tungsten are given in Table 3-19.

TABLE 3-19. RELATIVE SPECTRAL ENERGY DISTRIBUTION OF A STANDARD 500-W TUNGSTEN-FILAMENT LAMP WITH A COLOR TEMPERATURE OF 2854°K

Wave length, m $\mu$	Emissivity of tungsten at 2800°K	Relative energy for a complete radiator		Relative energy of a tungsten lamp at 2854°K ( $T_c$ ) (5) (2) $\times$ (3) $\div$ 0.44
		At 2800°K ( $T_f$ )	At 2854°K	
(1)	(2) <sup>a</sup>	(3) <sup>a</sup>	(4) <sup>a</sup>	
320	0.48	1.7	1.9	1.9
340	0.48	3.3	3.6	3.6
360	0.48	5.7	6.2	6.2
380	0.47	9.1	9.8	9.7
400	0.47	14	15	15
420	0.46	20	21	21
440	0.46	28	29	29
460	0.45	37	38	38
480	0.45	47	48	48
500	0.45	59	60	60
520	0.44	72	72	72
540	0.44	86	86	86
560	0.44	100	100	100
580	0.43	115	114	113
600	0.43	130	129	128
620	0.43	145	144	142
640	0.42	160	158	153
660	0.42	176	172	168
680	0.42	190	185	181
700	0.41	204	198	190
720	0.41	218	210	204
740	0.41	230	222	215
760	0.40	241	232	220
780	0.40	252	242	230
800	0.40	262	246	240
850	0.39	280	265	250
900	0.38	295	275	255
1000	0.37	310	283	260
1500	0.30	230	203	155
2000	0.23	134	117	69

<sup>a</sup> Literature references (at end of Sect. 2) for data: column 2, Forsythe and Adams (1945); column 3, Fowle (1929); column 4, Gibson (1949, p. 42) (reference at end of this section), Skogland (1929); see also Sect. 2 under Thermal Sources.

The data for the spectral energy distribution of the standard lamp given in Table 3-19 were obtained by multiplying the emissivity data (column 2) by the relative spectral intensity of a complete radiator at the true temperature of the filament (column 3) and arbitrarily reducing the values to 100 at 560  $m\mu$ . The relative spectral energy distribution for a complete radiator at 2854°K is given in column 4 for comparison. The spectral-emissivity data for tungsten are those of Forsythe and Adams (1945); similar data have been published by Ornstein (1936), but the two sets of values differ markedly in some regions (see Sect. 2 for references). It is the uncertainty as to the spectral emissivity of tungsten which limits the accuracy with which energy-distribution curves, obtained from color-temperature data, may be extrapolated into the ultraviolet and infrared. In general, however, the values given in Table 3-19 closely approximate those given by Forsythe and Adams for the direct measurement of a similar lamp of the same color temperature (2848°K on the 1931 temperature scale and 1854°K on the 1948 scale).

The temperature is not uniform throughout all parts of the filament of a lamp, especially where contact is made with the supports. The color temperature specified is the average for the whole filament and introduces a small error in the calculation of spectral energy distribution. In order to obtain a more uniform filament temperature for the emitted radiant energy and to be able to extrapolate spectral energy distribution further into the ultraviolet, Stair and Smith (1943) designed a special lamp in a quartz envelope, with a tungsten wire filament arranged in a series of four hairpin turns. The flux radiated beyond the lamp is limited to the straight portions of the filament by metal shields over the support wires and end turns. The color temperature of the exposed portions of the filament is very uniform and subject to more precise extrapolation than is possible for the glass-projection-lamp standard. Coolidge (1944) has studied the mercury arc as a standard of spectral energy distribution in the ultraviolet. Although this source is not so stable and reproducible as the incandescent lamp, its ultraviolet radiation is much more intense, and the lines can be isolated with filters.

#### SPECTROSCOPIC INSTRUMENTS

When comparing the performance of monochromators, it is often essential to have data on the spectral transmission of the complete instrument. If the monochromator is used with a detector to measure the spectral energy distribution of a source, the combination must be calibrated for spectral responsivity and accuracy of wave-length indication. Spectrophotometers must be occasionally calibrated for transmission and wave length (Gibson, 1949; Gibson and Balcom, 1947; Mellon, 1950; Normand and Kay, 1952). These operations require the use of standards of wave length, spectral energy distribution, and transmission.

*Wave Length.* The primary standard of wave length to which all other wave lengths and other physical-length measurements are referred is the red line of cadmium, which has a value of 6438.4696 Å. This value is known with a precision of 1 part in 10,000,000. A more convenient standard proposed by Meggers and Westfall (1950) consists of an electrode-less discharge lamp containing the single mercury isotope  $\text{Hg}^{198}$ , obtained from neutron bombardment of gold,  $\text{Au}^{197}$ . This isotope produces the usual mercury spectrum entirely free of the isotope shifts and hyperfine structure that characterize the mixture of isotopes present in natural mercury. A low-pressure discharge tube containing the mercury isotope is available from the National Bureau of Standards. The single green line at 5460.7532 Å may be isolated with filters.

Secondary standards of wave length are obtained from line sources in which the lines are sufficiently isolated for easy recognition (see Table 3-12). For the calibration of monochromators used for irradiation and spectrophotometry, the most useful standards are the mercury, sodium, cesium, and helium discharge tubes and flames to which lithium and potassium salts have been added. In Table 3-12 are given the principal lines of mercury, sodium, and helium. Discharge lamps for many other elements are available, as discussed in Sect. 2 under Amalgam Arcs and Alkali-metal Arcs. Cesium has two intense red lines at 852.1 and 894.4  $\text{m}\mu$ , which are especially useful since the mercury arc is deficient in the red. The accuracy of the wave-length calibration of recording spectrophotometers may be determined by making an absorption-spectrum curve of a standard didymium glass filter. The absorption bands of the rare earths are so sharp that the maxima make convenient wave-length reference points for recording spectrophotometers (Gibson, 1949; Mellon, 1950).

*Transmission and Spectral Energy Distribution.* The transmission of a monochromator may be determined by either of two methods: (1) by passing monochromatic energy through the instrument and measuring the ratios of energy passing through the entrance and exit slits, and (2) by measuring the transmitted energy from a source of known spectral energy distribution (Stair, 1951). With the second method the radiant flux from a lamp of known color temperature and spectral energy distribution is passed through the monochromator, and the emergent energy measured with a nonselective detector such as a thermocouple or bolometer. It is important that no condensing optics that will introduce spectral distortion be used with the color-temperature standard. A tungsten-filament lamp of known color temperature, such as the 2854°K color-temperature standard of the National Bureau of Standards, is well adapted to these measurements in the spectral range of 320–2000  $\text{m}\mu$ .

Stair (1951) has used the standard of color temperature to calibrate the over-all spectral response of a quartz double monochromator and

photomultiplier tube. Such a calibrated system is suitable for determining the relative spectral energy distribution of sources. The spectral energy distribution of the 2854°K color-temperature standard is given in Table 3-19.

*Absorption Standards.* Four relatively permanent glass standards of spectral transmittance and absorbance are available from the National Bureau of Standards as cobalt blue, copper green, carbon yellow, and selenium orange. Each filter standard is supplied with a certificate giving the transmittance and absorbance at various wave lengths. Typical transmittance curves are given by Gibson (1949).

For most applications, however, it is satisfactory to use solution standards that can be prepared in any laboratory. The composition of three such solutions and their transmittancies in 10-m $\mu$  steps are given by Gibson (1949) and Haupt (1952). An abridged form of these data is presented in Table 3-20 for solutions of copper sulfate for the red, cobalt sulfate for the green and blue, and potassium chromate for the ultraviolet. These solutions may be used for the calibration of the absorbancy (density) transmittance scales and as a secondary means of checking wave length. If the instrument closely reproduces the values given in Table 3-20, it can generally be assumed that the wave-length calibration is satisfactory. Transmittancy values are conveniently converted to absorbancy by the use of the data in Table 3-21.

#### VOLTAGE REGULATORS

Random voltage fluctuations in power services often make necessary the use of automatic voltage or current regulators, either as separate devices placed ahead of the regulated equipment or incorporated as an integral part of the instrument. When they are used as separate devices, it is important to consider the basic limitations of the various types available. Regulators for use in the United States on 60-cps 115-v service are usually\*designed for a supply variation of 95-115 v. Most of the regulators fall into one of the following classes: (1) electromechanical motor-controlled variable transformers, (2) magnetic resonant transformers, and (3) those employing electronic or magnetic amplifiers. The electromechanical regulators frequently have a servomotor that operates the contacts on a variable transformer. The reversible motor is coupled to a voltage-sensing device and amplifier. This type is usually employed only in high-power applications. It introduces little harmonic distortion but is relatively slow as compared with the other types.

The magnetic-resonant-transformer type of regulator contains separate primary and secondary windings and capacitors for producing resonance to the power frequency. These regulators are rugged and require no maintenance; the response time is less than 0.1 sec; and they are available in a wide range of output voltages. Since they operate on the

TABLE 3-20. SPECTRAL ABSORBANCY  $A$  AND TRANSMITTANCY  $T$  OF STANDARD SOLUTIONS FOR THE CALIBRATION OF SPECTROPHOTOMETERS<sup>a</sup>

Wave length, $m\mu$	Potassium chromate <sup>b</sup>		Wave length, $m\mu$	Cobalt sulfate <sup>c</sup>		Copper sulfate <sup>d</sup>	
	$A$	$T$		$A$	$T$	$A$	$T$
210	.....	0.000	360	0.0040	0.991	0.0063	0.986
220	0.446	0.358	370	0.0050	0.989	0.0046	0.989
225	0.221	0.601	380	0.0065	0.958	0.0035	0.992
230	0.171	0.674	390	0.0088	0.980	0.0028	0.994
235	0.210	0.616	400	0.0125	0.972	0.0023	0.995
240	0.295	0.507	410	0.0168	0.962	0.0019	0.996
245	0.396	0.402	420	0.0224	0.950	0.0016	0.996
250	0.496	0.319	430	0.0340	0.925	0.0014	0.997
255	0.572	0.268	440	0.0522	0.887	0.0012	0.997
260	0.633	0.233	450	0.0773	0.837	0.0011	0.997
265	0.695	0.202	460	0.1031	0.789	0.0011	0.997
270	0.745	0.180	470	0.1213	0.756	0.0012	0.997
275	0.757	0.175	480	0.1349	0.733	0.0014	0.997
280	0.712	0.194	490	0.1472	0.713	0.0018	0.996
285	0.590	0.257	500	0.1635	0.686	0.0026	0.994
290	0.428	0.373	510	0.1742	0.670	0.0038	0.991
295	0.273	0.533	520	0.1689	0.678	0.0055	0.987
300	0.149	0.709	530	0.1452	0.716	0.0079	0.982
305	0.079	0.834	540	0.1113	0.774	0.0111	0.975
310	0.048	0.895	550	0.0775	0.837	0.0155	0.965
313	0.043	0.905	560	0.0496	0.892	0.0216	0.951
320	0.064	0.864	570	0.0308	0.932	0.0292	0.935
330	0.149	0.710	580	0.0207	0.953	0.0390	0.914
340	0.316	0.483	590	0.1058	0.964	0.0518	0.888
350	0.559	0.276	600	0.0137	0.969	0.0680	0.855
360	0.830	0.148	610	0.0124	0.972	0.0885	0.816
370	0.987	0.103	620	0.0115	0.974	0.1125	0.772
380	0.932	0.117	630	0.0112	0.975	0.143	0.719
390	0.695	0.202	640	0.0110	0.975	0.180	0.661
400	0.396	0.402	650	0.0105	0.976	0.224	0.597
410	0.199	0.632	660	0.0097	0.978	0.274	0.532
420	0.124	0.751	670	0.0087	0.980	0.332	0.466
430	0.084	0.824	680	0.0076	0.983	0.392	0.406
440	0.054	0.882	690	0.0066	0.985	0.459	0.348
450	0.033	0.927	700	0.0054	0.988	0.527	0.297
460	0.018	0.960	710	0.0046	0.989	0.592	0.256
470	0.009	0.980	720	0.0038	0.991	0.656	0.221
480	0.004	0.991	730	0.0032	0.993	0.715	0.193
490	0.001	0.997	740	0.0030	0.993	0.768	0.171
500	0.000	1.000	750	0.0028	0.994	0.817	0.152

<sup>a</sup> Path length, 1 cm; temperature, 25°C.<sup>b</sup> Potassium chromate ( $K_2CrO_4$ )

Potassium hydroxide (85% KOH)

Water to make

<sup>c</sup> Cobalt sulfate ( $CoSO_4 \cdot 7H_2O$ )

Sulfuric acid (specific gravity 1.835)

Water to make

<sup>d</sup> Copper sulfate ( $CuSO_4 \cdot 5H_2O$ )

Sulfuric acid (specific gravity 1.835)

Water to make

0.040 g

3.3 g

1000 ml

10.3 g

10.0 ml

1000 ml

20.0 g

10.0 ml

1000 ml

TABLE 3-21. CONVERSION OF TRANSMITTANCE  $T$  TO ABSORBANCE,  $-\log T$ 

$T$	$-\log T$	$T$	$-\log T$	$T$	$-\log T$
1.00	0.0000	0.65	0.1871	0.25	0.602
0.995	0.0022	0.64	0.1938	0.24	0.620
0.99	0.0044	0.63	0.2007	0.23	0.638
0.985	0.0066	0.62	0.2076	0.22	0.658
0.98	0.0088	0.61	0.2147	0.21	0.678
0.975	0.0110	0.60	0.2219	0.20	0.699
0.97	0.0132	0.59	0.2292	0.19	0.721
0.965	0.0155	0.58	0.2366	0.18	0.745
0.96	0.0177	0.57	0.2441	0.17	0.770
0.955	0.0200	0.56	0.2518	0.16	0.796
0.95	0.0223	0.55	0.2596	0.15	0.824
0.94	0.0269	0.54	0.2676	0.14	0.854
0.93	0.0315	0.53	0.2757	0.13	0.886
0.92	0.0362	0.52	0.2840	0.12	0.921
0.91	0.0410	0.51	0.2924	0.11	0.959
0.90	0.0458	0.50	0.3010	0.10	1.000
0.89	0.0506	0.49	0.3098	0.09	1.046
0.88	0.0555	0.48	0.3188	0.08	1.097
0.87	0.0605	0.47	0.3279	0.07	1.155
0.86	0.0655	0.46	0.3372	0.06	1.222
0.85	0.0706	0.45	0.3468	0.055	1.260
0.84	0.0757	0.44	0.3566	0.05	1.301
0.83	0.0809	0.43	0.3665	0.045	1.347
0.82	0.0862	0.42	0.3768	0.04	1.398
0.81	0.0915	0.41	0.3872	0.035	1.456
0.80	0.0969	0.40	0.3979	0.03	1.523
0.79	0.1024	0.39	0.4089	0.025	1.602
0.78	0.1079	0.38	0.4202	0.02	1.699
0.77	0.1135	0.37	0.4318	0.015	1.824
0.76	0.1192	0.36	0.4437	0.01	2.000
0.75	0.1249	0.35	0.4559	0.010	2.000
0.74	0.1308	0.34	0.4685	0.009	2.046
0.73	0.1367	0.33	0.4815	0.008	2.097
0.72	0.1427	0.32	0.4949	0.007	2.155
0.71	0.1487	0.31	0.5086	0.006	2.222
0.70	0.1549	0.30	0.5229	0.005	2.301
0.69	0.1612	0.29	0.5376	0.004	2.398
0.68	0.1675	0.28	0.5528	0.003	2.523
0.67	0.1739	0.27	0.5686	0.002	2.699
0.66	0.1805	0.26	0.5850	0.001	3.000

principle of electrical resonance, it is essential that the supply frequency be very constant. For one commonly used type a 1 per cent change in frequency causes a 1.8 per cent change in output voltage in the same direction as the frequency change. In the large interconnected power systems of continental America, the average frequency is very constant, as evidenced by the precision of electrical clocks. However, there are short-time variations that may be as large as  $\pm 0.25$  cps or 0.4 per cent. This limits the short-time regulation accuracy to  $\pm 0.7$  per cent, although the long-time regulation will be very much better. Standard regulators are usually designed to reduce a  $\pm 17$  per cent supply fluctuation to  $\pm 1$  per cent for a constant load. By operating two regulators in cascade so that the first regulates the power supplied to the second, the average long-time variation in voltage can be reduced to less than  $\pm 0.1$  per cent, but there is little improvement in short-time stability. When regulators with electronic devices employing rectifiers are used, it is important that the low-harmonic-distortion type be employed. The regulator supplies constant root-mean-square voltage, whereas the voltage output from a rectifier supplying a capacitor input filter is dependent on the peak value of the voltage wave. The low-harmonic-distortion types are larger and more costly, but they produce a pure sine wave relatively free of third-harmonic distortion.

The electronic a-c regulators employ a saturable reactor type of transformer controlled by a voltage-sensing element on the output and an amplifier. One type has a diode whose filament temperature is dependent on the output voltage. A change in filament emission causes a voltage change which is amplified and controls the reactance of the power transformer in such a way as to oppose the change. These regulators have the important advantage that they are not power-frequency-dependent and are available with regulation control of 0.1–0.001 per cent. The harmonic distortion is low, but the speed of regulation is not quite so rapid as that of the magnetic regulators.

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## Energy Efficiency in Photosynthesis

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*The process of photosynthesis. Principles of photochemistry. Measurement of energy. Measurement of chemical change in photosynthesis by nonmanometric methods. Measurement of chemical change in photosynthesis by manometric methods. Biological factors. Practical applications. Discussion. References.*

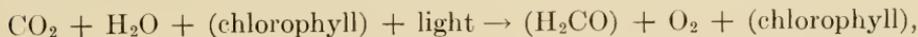
What is the theoretical maximum utilization of solar energy in growing plants? This is an important question both in the laboratory, where different mechanisms for photosynthesis are proposed, and in the economic world, where geographical agricultural expansion is reaching its limit and where the exhaustion of fuel reserves is foreseeable.

Any proposed mechanism for photosynthesis must fall within the energy balance of the observed over-all process. A low maximum efficiency of energy conversion will permit a wide variety of exothermic reaction steps in the photosynthetic process. On the other hand, if the conversion of radiant energy into chemical energy is very high, 70 per cent, for example, only a limited number of exothermic reactions can be fitted into the photosynthetic mechanism.

Different workers in the field of photosynthesis do not agree concerning the maximum efficiency of energy storage in photosynthesis, and it is the purpose of this chapter to examine the various ways of measuring the energy absorbed and the chemical products in photosynthesis.

### 1. THE PROCESS OF PHOTOSYNTHESIS

The primary reaction of photosynthesis is given by the equation



where  $(\text{H}_2\text{CO})$  represents a unit of a carbohydrate associated with a gram-atom of carbon. At the time of this writing (1952) it has not been possible to produce this reaction in the absence of chlorophyll or in any way outside the living plant, but there seems to be no theoretical reason why photosynthesis cannot at some time be accomplished *in vitro* as well as *in vivo*.

As indicated by the chemical equation, one molecule of carbohydrate is formed in the growing plant while one molecule of carbon dioxide disappears from the surrounding gas and one molecule of oxygen is formed. However, plant material is not composed entirely of carbohydrates. Fats and proteins are produced as well, and the conditions can be changed so as to vary greatly the ratios of these basic organic materials (Spoehr and Milner, 1949). Only in the production of carbohydrates will the ratio of the molecules of carbon dioxide consumed to those of oxygen evolved, defined as  $\gamma$ , be exactly  $-1$ . Sometimes the carbon dioxide may not be carried all the way down to carbohydrates in the reduction by the hydrogen released photochemically from water in the presence of chlorophyll. If acids or other partially reduced products of carbon dioxide are formed, the value of  $\gamma$  is not unity and the amount of oxygen released is not equal to the carbon dioxide consumed. Methods of measuring the efficiency of photosynthesis which depend on adherence to the equivalence of oxygen and carbon dioxide (i.e., to the relation  $\gamma = -1$ ) may be subject to error. They require constant checking.

The photosynthetic efficiency depends on many factors. In order to determine the maximum efficiency, it is necessary that light energy be the limiting factor. To be sure that this is the case, the light intensity must be kept low, the carbon dioxide concentration high, the temperature high but not too high, and the supply of water, carbon dioxide, chlorophyll, and inorganic nutrients ample. These relations are fundamental in any study of photosynthesis. They are described in terms of the Blackman curve, according to which, at low light intensities, the amount of photosynthesis is directly proportional to the intensity of the light, or, in other words, the amount of photosynthesis per unit of light absorbed is a constant. At high light intensities a "saturation" is reached such that a further increase in the light intensity does not lead to an increase in the rate of photosynthesis. The thermal and biological reactions that follow the primary photostep cannot keep up with it. Significant measurements of energy efficiencies should be made under conditions below saturation, where the amount of photosynthesis doubles when the intensity of light is doubled. The complexity of photosynthesis is indicated by the classic research of Emerson and Arnold (1932), in which it was shown that increased light efficiency can be obtained with flashing light, the rest period enabling the thermal reactions to accumulate intermediate products.

## 2. PRINCIPLES OF PHOTOCHEMISTRY

According to the well-established principle of Grotthus, only that light which is absorbed can be chemically active. That part of a light beam which passes through a substance unabsorbed is without chemical effect.

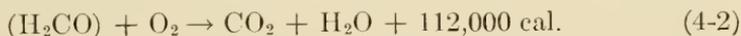
Another fundamental law of photochemistry accepted by scientists is that each unit, or photon, of light absorbed can activate one molecule. This one-to-one relation, which was established by Einstein, refers to the number of molecules *activated* by the light, and not to the number of molecules reacting chemically. A large number of different secondary reactions can follow the primary activation of the absorbing molecule.<sup>1</sup>

Ordinary chemical reactions obtain the large activation energies that they need from collisions between rapidly moving molecules, and these violent, chemically significant collisions increase greatly with an increase in the temperature of the reacting system. In photoactivation, however, the energy of activation of the primary step comes from light from an outside source and is not influenced appreciably by the temperature of the reacting system. The secondary, thermally activated reactions can, of course, be changed by changing the temperature. The extent to which an over-all photochemical reaction, or photosynthesis, depends on temperature offers a means for distinguishing between the thermal and photochemical steps. Inasmuch as photosynthesis involves secondary thermal reactions, better efficiencies are obtained at higher temperatures, provided they are not so high as to destroy some of the biological processes.

It is helpful to consider the minimum energy requirements for photosynthesis. The primary reaction of photosynthesis,



can be reversed. When a carbohydrate, represented by  $\text{H}_2\text{CO}$ , is burned in oxygen to give carbon dioxide and water, heat is evolved, as determined with a combustion calorimeter, amounting to 112,000 cal per mole of  $6.02 \times 10^{23}$  molecules. The reverse reaction is written



By a well-known principle of thermochemistry, it is necessary to supply at least as much as the 112,000 cal in order to make the reverse of reaction (4-1) take place. In fact, it will be necessary to supply more than the 112,000 cal, because an energy of chemical activation must be supplied in addition to the thermodynamic heat of reaction in order to make the reaction go with measurable speed. Accordingly, if carbohydrates are to be produced from carbon dioxide and water by the absorption of light, the light must be energetic enough to be the equivalent of more than 112,000 cal/mole.

The energy contained in light is easily calculated from the quantum theory, according to which the energy of one unit of radiation, 1 photon,

<sup>1</sup> These secondary reactions are ordinary thermal reactions governed by the laws of thermodynamics and chemical kinetics. Early workers sometimes misinterpreted Einstein's law of photochemistry to indicate that there should be one molecule of *product* for each photon absorbed.

is given by the relation  $E = h\nu$ , where  $E$  is the energy in 1 photon, called a quantum;  $h$  is Planck's constant,  $6.56 \times 10^{-27}$  erg-sec; and  $\nu$  is the frequency of light per second. In the case of red light having a wave length of 7000 Å, or  $7 \times 10^{-5}$  cm, the frequency is obtained by dividing the velocity of light,  $3 \times 10^{10}$  cm/sec, by the wave length,  $7 \times 10^{-5}$  cm, to give a frequency of  $4.3 \times 10^{14}$  per second. The physical chemist carries out his calculations in terms of moles of  $6.02 \times 10^{23}$  molecules rather than single molecules, and the energy of the light for a mole is obtained by multiplying the energy of 1 photon by  $6.02 \times 10^{23}$  and converting this to calories by means of the relations  $10^7$  ergs = 1 joule and 4.18 joules = 1 cal. The energy of red light is thus

$$\frac{6.02 \times 10^{23} \times 6.56 \times 10^{-27} \times 4.3 \times 10^{14}}{4.18 \times 10^7} = 40,500 \text{ cal/mole.}$$

By similar calculations, blue light of 4000 Å furnishes 71,500 cal/mole.

It is clear that photosynthesis calls for energy of high intensity, at least as high as 112,000 cal/mole, whereas red light of wave length 7000 Å furnishes only 40,500 cal/mole. This is only about one-third enough. Photosynthesis, however, certainly takes place very effectively in red light, and a mechanism must be found by which the energy of at least 3 photons can be utilized for each molecule of carbon dioxide consumed and oxygen evolved. If any steps are less than 100 per cent efficient energy-wise, if activation energies are required, or if exothermic reactions are involved, the photosynthesis with red light will require more than 3 photons per molecule.

Although 3 photons together theoretically contain enough energy to effect photosynthesis, it is highly improbable that 3 or more photons can collide simultaneously with a molecule of carbon dioxide and a molecule of water. Thermodynamically 3 or more are required, but the mechanism of the utilization of the energy is uncertain. Successive reduction by hydrogen released from water is now considered to be a likely mechanism. This is discussed fully in other chapters of this volume.

In actual photosynthesis it is to be expected that more than the minimum of 112,000 cal will be required. The relation between energy absorbed and plant product is calculated and reported in two different ways: the quantum yield and the energy efficiency.

The *quantum yield*  $\Phi$  is defined in photochemistry as the number of molecules undergoing chemical change per photon or per quantum absorbed:

$$\Phi = \frac{\text{No. of molecules undergoing chemical change}}{\text{No. of photons absorbed}}$$

If the primary activation of one molecule by a photon is followed by a chemical reaction that involves two molecules, the quantum yield is 2.

If 4 photons are required to produce the reaction of one molecule,  $\Phi = \frac{1}{4}$ . In photosynthesis it has become the habit to refer to the reciprocal of the quantum yield:

$$\frac{1}{\Phi} \text{ or } \Phi^{-1} = \frac{\text{No. of photons absorbed}}{\text{No. of molecules in chemical change}}$$

This ratio has been given the name *quantum requirement*. Thus, if the quantum yield is 0.1 molecule per photon, the quantum requirement is 10 photons per molecule.

The energy efficiency in photosynthesis is defined as follows:

$$\text{Energy efficiency} = \frac{\text{theoretical minimum energy}}{\text{experimentally determined energy}}$$

If 3 photons of red light should ever be found capable of producing a molecule of photosynthetic product, the energy efficiency would be equal to  $112,000/(3 \times 40,500)$ , or 0.92. If 3 photons of blue light at 4000 Å are required, the energy efficiency is  $112,000/(3 \times 71,500)$ , or 0.52.

If 4 photons of red light at 7000 Å are required, the energy efficiency is  $112,000/(4 \times 40,500)$ , or 0.70. And if 8 is required, the efficiency is 0.35.

### 3. MEASUREMENT OF ENERGY

In photochemical research it is desirable to use essentially monochromatic light because all the photons then have the same amount of energy, the photoreactions are sure to be the same, and the calculations are made easier. Chlorophyll has strong absorption bands in the red and in the blue, but it absorbs also to a limited extent throughout most of the visible spectrum, from 4000 to 7000 Å.

It happens that in photosynthesis the quantum yield is about the same in blue, green, yellow, and red light, but it is desirable, nevertheless, to use monochromatic light. A discontinuous source of light, such as a mercury-vapor lamp (Daniels *et al.*, 1949; Rabinowitch, 1951) or a cadmium arc combined with a prism or grating, gives the best source of light. Light filters (Daniels *et al.*, 1949) of colored glass or interference filters of thinly deposited metals can be used. Red light, with its lower energy per photon, is used most commonly because with it greater energy efficiency can be expected. However, metallic-vapor lamps giving red light are difficult to operate, and most of the work in the red has been done with tungsten lamps and optical monochromators (*ibid.*) or filters.

The energy of the light in ergs per second per square centimeter is usually measured with thermopiles or bolometers calibrated against a standard carbon-filament lamp supplied by the U.S. National Bureau of Standards (*ibid.*). When the surfaces are properly blackened, the bolometer or the thermopile gives equal deflections on a sensitive galvanometer

per erg of radiant energy received, irrespective of the wave length of the light. Energies from 500 to 50,000 ergs/sec/cm<sup>2</sup> cover the range of ordinary experimentation in photosynthesis. Bright sunlight has an energy of over  $5 \times 10^5$  ergs/sec/cm<sup>2</sup>.

Although the calibrated thermopile and bolometer are accepted as the primary standards in energy measurements of photosynthesis, chemical actinometers are often more convenient and sometimes more suitable. The uranyl oxalate actinometer (*ibid.*) is the best chemical actinometer, but it is responsive only to blue and to ultraviolet light. The amount of oxalate decomposed by the light is measured by titration with potassium permanganate, 0.57 molecule being decomposed by each photon of light absorbed. It is useful in checking the calibration of any radiation-recording instrument, but its absorption spectrum is so different from that of chlorophyll that it cannot be used directly in photosynthesis.

The ethyl chlorophyllide actinometer, developed by Warburg, Burk, and Schade (1951), Warburg and Schocken (1949), and Gaffron (1927), has also been studied by Evans (1951). It is ideally suited for energy measurements in photosynthesis because its absorption is nearly the same as that of chlorophyll. The actinometer as developed by Warburg and Schocken (1949) is read by measuring the consumption of oxygen in the same type of Warburg manometer used for measurements of respiration and photosynthesis. The light absorber is about 2 mg of ethyl chlorophyllide extracted by a special process from leaves of the stinging nettle, dissolved in 7 ml of pyridine containing 200 mg of allyl thiourea.

The quantum yield  $\Phi$  was determined by Evans (1951) under a variety of conditions and found to average 0.96 molecule of oxygen consumed in the solution per photon absorbed when the solution is shaken (150 times per minute) in a Warburg manometer. There is a slight dependence on the intensity of light. The use of this actinometer in light transmission measurements by Warburg and Burk is a distinct advance.

A serious problem in measurements of photosynthesis is the scattering of light by algae. If a beam of light is passing directly through a clear aqueous solution onto a receiver, this light beam will be altered by the introduction of a suspension of algae. Some of the light will be absorbed, and some of the light that is not absorbed will be scattered. The more concentrated the suspension of algal cells, the longer the path of the light, and the smaller the size of the algal cells, the greater the amount of the scattering. If the thermopile or bolometer receiver is considerably larger in area than the normal beam, most of the scattered light energy will still be registered, because the wide-angle scattering of light is less probable than the scattering of light that departs only slightly from the main beam and still falls on the large-area thermopile. In the case of a large-area photocell and receiver, there is a tendency, too, for some of the scattered light to be scattered back again. The closer the large-area

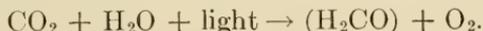
receiver is placed to the back of the photocell, the smaller will be the scattering losses.

The scattering of light by algal suspensions has been studied also by Emerson and Lewis (1942) and by Kok (1948). Probably it was the difficulty of allowing for the scattered light which led Warburg, in his early work with Negelein (Warburg and Negelein, 1923) and in much of his later work, to use such a high concentration of algal cells that the light was totally absorbed. All the incident light is then used in photosynthesis. But in this procedure the price paid for the precise measurement of energy is high, because the large excess of algal cells causes respiration to overbalance photosynthesis, and the algal cells are being exposed to light of all different intensities from full intensity at the front of the solution to zero at the back. Moreover, if the photocell is shaken, the algal cells are exposed to varying and uncertain alternating periods of light and dark which can complicate the relation between the primary photoprocess and the thermal reactions that follow.

The light-scattering difficulty, which is most serious in concentrated solutions, can be solved with the help of an integrating reflecting Ulbright sphere, which catches all light that enters in. The glass cell in which photosynthesis takes place is set in the center of a large internally reflecting sphere, which also contains the thermopile or other energy-measuring instrument. Light is admitted to the reaction chamber through a hole in the sphere. Calibrations may be made with the cell filled with totally absorbing ink solution, and with a reflecting suspension of calcium carbonate having particles of the same size as the algal cells. Rieke (1939) and Kok (1948) have used this method successfully.

#### 4. MEASUREMENT OF CHEMICAL CHANGE IN PHOTOSYNTHESIS BY NONMANOMETRIC METHODS

Equally as important as the measurement of the energy consumption is the measurement of the chemical changes brought about by photosynthesis in the reaction



Obviously there are many different ways in which changes in these four chemical compounds can be measured by chemical or physical means so as to follow the progress of the reaction, remembering always that the reverse reaction in the form of respiration is going on continuously in the living material whether or not photosynthesis is taking place.

The exact measurement of chemical change is handicapped by the fact that the amount of the change is usually very small. In most of the investigations of energy requirements thus far, it has been the aim to obtain as high an efficiency as possible, and the experiments have been

of short duration, in easily handled laboratory equipment using monochromatic light. But precise measurements with monochromatic light require that the intensity of radiation be low, because the monochromators or filters reduce the light intensity. High biological efficiency also requires that the intensity of the light be low. The energy can be measured precisely, but the chemical changes are so small that micro methods must be used, and micro methods are apt to give results of low accuracy. Much ingenious work has gone into the perfecting of micro methods for determining the chemical change in photosynthesis.

The most obvious way in which to follow photosynthesis and respiration is to measure the change in concentration of oxygen and carbon dioxide of the surrounding gas by standard methods of gas analysis. The micro gas analysis for carbon dioxide and oxygen using alkaline absorbents to remove the carbon dioxide and phosphorus or alkaline pyrogallie acid to remove the oxygen has been perfected (Manning, Stauffer, *et al.*, 1938). The analysis can be carried out either in a flow system in which the gases are absorbed and weighed or in an enclosed space in which the gases are removed by absorption and measured volumetrically. However, the changes in composition of the gases in most experiments of photosynthesis are so small that this method has not been much used.

The chemical determination of carbohydrates or of carbon dioxide is difficult in a complex system such as a suspension of algae or a mass of growing plants, but the chemical analysis for dissolved oxygen is quite simple. The Winkler method, based on the oxidation of manganous hydroxide by dissolved oxygen and subsequent titration for iodine, is simple, and it has been used successfully (Magee *et al.*, 1939; Petering and Daniels, 1938; Petering *et al.*, 1939). The algal suspension is exposed to the measured light in square bottles with polished sides and titrated under conditions that prevent errors from atmospheric oxygen during analysis. Good results have been obtained even when the algae are present in the titrating solution.

The dissolved oxygen can be determined more easily by means of the dropping-mercury electrode (Petering and Daniels, 1938; Petering *et al.*, 1939; Dutton and Manning, 1941) by an empirical method in which the differences in galvanometer current at 1.0 and 0.1 volt are measured and converted to oxygen concentration by reference to a calibration curve in which the currents are plotted against known concentrations of dissolved oxygen, conveniently determined by the Winkler method. The mercury cathode is kept separate from the mercury that drops down from the capillary anode. Experiments have shown that the metallic mercury has no detrimental physiological effect on *Chlorella*, although some complication was observed in experiments with salt-water diatoms. The response to dissolved oxygen is rapid, with a time lag of less than half a

minute. It is not necessary to get the dissolved oxygen out of the solution into the gas phase, as is the case with gas analysis or manometric methods. The electrode is placed in the center of the beam of light, and stirring is not essential.

Oxygen can be determined polarigraphically also with a platinum anode as well as with the dropping-mercury electrode. Brackett (Olson, Brackett, and Crickard, 1949) has developed a very effective electronic circuit which he uses for the analysis of oxygen in algal suspensions with a time lag of less than 10 sec.

The storage of energy in the process of photosynthesis can be determined calorimetrically (Arnold, 1949; Magee *et al.*, 1939; Tonnelat, 1944). The number of ergs of radiation absorbed by the algal suspension is determined in a small glass cell with clear flat windows at front and back. A thermopile is placed back of the cell, and the energy passing through the cell is measured first with the clear nutrient solution filling it and then again after it is filled with algae. Multiple thermocouples are placed all around the photocell so that a slight increase in temperature of the algal suspension can be measured. In this way the photocell acts also as a microcalorimeter. It is calibrated with an opaque solution of india ink or other inert material. When the heat evolved in the cell is subtracted from the amount of radiant energy absorbed, a measure is obtained of the amount of energy stored as chemical energy in the form of carbohydrates or other plant material. The percentage of energy stored chemically can then be calculated in terms of photons per molecule. Stirring of the algae is difficult in these calorimetric experiments, and the dissolved oxygen and carbon dioxide must be relied upon to supply the necessary chemicals for photosynthesis and respiration. This calorimetric method offers an independent means for determining the energy conversion in photosynthesis.

Photosynthesis leads to growth of plants and to an increase in the number of algal cells. The increase in dry weight following synthesis should be a direct measure of the amount of photosynthesis. For the determination of the total amount of energy stored in photosynthesis, the heat of combustion as measured in a bomb calorimeter would seem to offer an excellent method. One gram of material is sufficient for determination of the heat of combustion, but this method has not yet been used. Went (1950) determined the increase in dry weight of tomato plants grown under optimum conditions. The light absorption was estimated, and the best conditions, involving low temperature during illumination and higher temperatures during dark periods, were determined empirically. He found, on the basis of the dry weight of the tomato plants and the calculated heat of combustion, that a maximum of about 20 per cent of the radiant energy absorbed could be stored chemically in the plants. This amounts to a quantum yield of about one molecule per 15 photons

for red light. Past researches have stressed the importance of determining the maximum yield, and it is unlikely that optimum conditions can be maintained long enough to grow many grams of new plant material. But more attention could well be paid to the direct weighing of the products of photosynthesis.

Possibly, between the micro scale of the laboratory and the macro scale of the "yield per acre" of agricultural crops, there may be a fruitful area for experimental studies of photosynthetic efficiencies. The growth of gram quantities of algae in tanks with suitable actinometers should be attractive (Geoghegan, 1951).

Oxygen is paramagnetic, whereas most other common gases are slightly diamagnetic. This property has been utilized by Pauling *et al.* (1946) for determining the oxygen concentration in a gas. A convenient and practical instrument has been developed by Beckman in which gas is passed through a chamber containing a light, dumbbell-shaped test body which is suspended by a fine quartz fiber in a strong, nonhomogeneous permanent magnetic field. A mirror attached to the fiber deflects a beam of light onto photocells. Then an electronic circuit is affected in such a way as to give a voltmeter reading that is directly proportional to the oxygen concentration of the gas passing through the magnetic field. This instrument has been found very satisfactory for measuring the oxygen changes in a circulating-gas system that involves photosynthesis or respiration. Its accuracy can be about the same as that of a Warburg manometer, and the reading is specific for oxygen.

The concentration of carbon dioxide in a gas stream can be determined by infrared absorption. Carbon dioxide has a distinctive absorption band at  $4.3 \mu$  in the infrared, which was used by McAlister (1937) in studies of photosynthesis. Evans (1951) has used this method for determining the quantum yield in photosynthesis.\* This infrared absorption method of analysis works very well for low concentrations of a few hundredths or tenths of a per cent, but it calls for a good deal of care in operating above 1 per cent carbon dioxide, which is the interesting range for investigations of photosynthesis. This infrared method is valuable because it is specific for carbon dioxide in a mixture with oxygen and nitrogen. The water vapor can be removed with absorbents.

The electrical conductance of a solution has been used as a measure of the carbon dioxide present under certain conditions. This method has been described and perfected by Wolf *et al.* (1952).

The mass spectrometer offers a method for the simultaneous determination of oxygen and carbon dioxide. It has been developed and used successfully by Brown *et al.* (1952). It can be used equally well in tracer experiments with isotopes.

\* Added in proof: An infrared gas analyzer by Grubb Parsons gives good results as reported by Yuan (1954, 1955).

In all methods of analysis it must be remembered that what is really desired is the carbon dioxide and oxygen concentration within the biological material. These concentrations may be different in the solution in which algal cells are suspended than they are in the gases bubbling through the liquid phase. All methods that analyze the gases call for a complete chemical equilibrium between the gas and the liquid phase, and care must be taken to see that this equilibrium is reached rapidly.

#### 5. MEASUREMENT OF CHEMICAL CHANGE IN PHOTOSYNTHESIS BY MANOMETRIC METHODS

The manometric method depends on the fact that carbon dioxide is more soluble in water than oxygen is. In photosynthesis an oxygen molecule is exchanged for a carbon dioxide molecule, and in respiration an oxygen molecule is consumed within the cell and a carbon dioxide molecule is liberated. If the volume is kept constant, there will be an increase in pressure when the more soluble carbon dioxide is replaced by the less soluble oxygen. If the solution is alkaline, the carbon dioxide will be completely dissolved in the solution, and the changes in gas pressure will be due to changes in oxygen alone.

There is an inherent danger in any method of analysis which is not specific for the chemical compound being determined. The manometer records an increase in pressure whenever additional molecules of any kind are introduced into the gas space. The manometer does not distinguish between oxygen and carbon dioxide or even nitrogen or methane, whereas chemical methods and some physical methods, such as infrared absorption, do. Physical chemists during the past have made serious errors in some researches on chemical kinetics by following the rate of the chemical reaction in the gas phase through measurements of the total gas pressure. If one molecule decomposed into two molecules, the pressure doubled, and if the reaction really went as assumed, the measurements were significant. But sometimes the reaction did not go as assumed—competing reactions used up the reactants in such a way that the pressure did not double when the reaction was complete. Measurements of total pressure in a gas mixture can be relied upon for determining the concentration of a single gaseous substance only when it is proved experimentally that the reaction in a specific case proceeds in the manner assumed in making the calculations.

Another danger in the manometric method in addition to this lack of specificity is the complication of the time factor. All calculations for the concentration of carbon dioxide and oxygen in the solution based on measurements of gas pressure involve the assumption that an equilibrium exists. But time is required for establishment of this equilibrium, and if measurements are made during the transient period, when equilibrium

conditions do not exist, serious errors may result. In measurements of photosynthesis the intensity of light is changed, or the conditions of dark and light are alternated. With each change there will be a change in concentration of carbon dioxide and oxygen which requires time for adjustment.

In one experiment (Evans, 1951; Inouye, 1951) to test the time lag directly, the manometers were allowed to reach equilibrium, and then a little carbon dioxide was introduced into the manometer vessel by pressing the plunger of a hypodermic syringe. At the shaking rate of 150 oscillations per minute used by Warburg and his associates, it took 30 sec to approach equilibrium conditions again within the limits of the accuracy of the experiments. It took 200 sec when the shaking was reduced to half the rate.

Oxygen reaches equilibrium much faster than carbon dioxide, and serious errors of interpretation can arise if measurements are taken before equilibrium of *both* gases is reached. The tendency has been to use short periods of light exposure, so that the biological conditions will not have time to change very much, and this emphasis on short exposures magnifies any error resulting from lack of equilibrium following a change of conditions. The time required for the attainment of equilibrium conditions varies greatly from perhaps 0.5 to 2 or 3 min, and it should be determined for each type of experiment. Warburg, Burk, and Schade (1951) urged a period of 5 min to remove the lag in one carbonate buffer solution.

The use of alkaline carbonate buffers simplifies the experimental conditions and the calculations, but even here the time factor can introduce complications. Carbon dioxide reaches equilibrium with carbonate solutions very slowly. In tissues and cells the enzyme carbonic anhydrase is known to accelerate this reaction. A buffer frequently used contains 85 parts of 0.1 *M* sodium bicarbonate freshly mixed with 15 parts of 0.1 *M* sodium carbonate. This mixture has a pH of about 9 and at 20°C is in equilibrium with an atmosphere containing 0.5 per cent carbon dioxide. The carbon dioxide content of the gas has been assumed to be constant, and any change in pressure is due to oxygen. Equilibrium is reached slowly. From this oxygen change it is possible to calculate the rate of photosynthesis and respiration. Some species of algae do not grow normally in carbonate buffers, but *Chlorella* cells seem to carry on normal photosynthesis for hours in alkaline buffers (Emerson and Lewis, 1941; Nishimura *et al.*, 1951). Warburg considered carbonate buffers "unphysiological" (Burk and Warburg, 1950) but apparently accepts them if the pH is under 8.8 (Warburg, Geleck, and Briese, 1951).

The ratio  $\gamma$  (see Sect. 1), which has a value of  $-1$  if the reaction proceeds strictly as  $\text{CO}_2 + \text{H}_2\text{O} \rightarrow (\text{H}_2\text{CO}) + \text{O}_2$ , is important in manometer calculations. The value of  $\gamma$  is best determined by direct chemical

analysis (Umbreit *et al.*, 1949; Warburg and Negelein, 1922), but it can be determined indirectly as described presently.

The oxygen change is calculated by the formula

$$O_2 = \frac{hk_{O_2}k_{CO_2}}{k_{CO_2} + \gamma k_{O_2}},$$

and the  $CO_2$  change is calculated by the formula

$$CO_2 = \frac{hk_{O_2}k_{CO_2}}{(1/\gamma)k_{CO_2} + k_{O_2}},$$

where  $h$  is the change in pressure and  $k_{O_2}$  and  $k_{CO_2}$  are the vessel constants obtained when only one gas is involved. The basic assumption of this method is that  $\gamma$  remains constant, but there is much evidence that it does not remain constant under the conditions used in the measurement of photosynthesis. Under certain conditions carbon dioxide is produced by exposure to light (opposed to photosynthesis), and this "Emerson carbon dioxide burst" produces an excess of carbon dioxide and gives an abnormal value of  $\Phi^{-1}$  when light is introduced into dark-conditioned algae (Emerson and Lewis, 1939, 1941, 1943; Nishimura *et al.*, 1951). Additional evidence for such a phenomenon has been supplied by the observation that increased acidity occurs upon illumination (Blinks and Skow, 1938).

Part of the burst is due to the sluggishness with which the carbon dioxide reaches equilibrium, and part is probably due to physiological conditions. Other workers believe that the value of  $\gamma$  does not change enough to affect their calculations (Warburg, 1948).

Carbon dioxide and oxygen can be determined simultaneously by the indirect method proposed by Warburg. This method has been very widely used. Two vessels are used which are alike except that the ratio of gas space to the volume of liquid is different in the two. The formula for the change in oxygen concentration is

$$\Delta O_2 = \frac{HK_{CO_2} - hk_{CO_2}}{K_{CO_2}/K_{O_2} - k_{CO_2}/k_{O_2}},$$

and that for the change in carbon dioxide concentration is

$$\Delta CO_2 = \frac{hk_{O_2} - HK_{O_2}}{K_{O_2}/K_{CO_2} - k_{O_2}/k_{CO_2}},$$

where  $k_{O_2}$ ,  $k_{CO_2}$ ,  $K_{O_2}$ , and  $K_{CO_2}$  are the vessel constants for the two vessels when only the indicated gas is exchanged. The symbol  $h$  is the manometer reading of pressure differences in the vessel for which the vessel constants  $k_{O_2}$  and  $k_{CO_2}$  apply, and  $H$  is the manometer reading for the other vessel, with the constants  $K_{O_2}$  and  $K_{CO_2}$ . A great many of the measurements of photosynthesis have been made by this method, as given in Table 4-1.

In one type of two-vessel manometric apparatus, the two vessels have the same cross section and the same exposure to light, and each is filled with algal suspension to the same depth. One vessel, however, has an extra gas space above the solution to change the (gas volume)/(liquid volume) ratio. In another type the vessels are identical, and the difference in gas/liquid ratio is accomplished by having the level of the algal suspension different in the two vessels. The latter arrangement, however, may give rise to a different amount of light absorption in the two vessels, particularly under the conditions of shaking, and thus lead to an inequality in the gas pressures of the two vessels which is not due to the different sizes. The two vessels containing the algal cells have optically perfect bottoms, through which the beam of light passes into the algal suspension. The light beams are adjusted until they are equal. The vessels are immersed in a water thermostat and connected to each other by a U-shaped manometer filled with water or other liquid of low density. The vessels and the manometer are shaken vigorously to expose a large surface of liquid to the gas in order to decrease the time required for the attainment of equilibrium. A shaking rate of 150 oscillations per minute and an amplitude of 1 or 2 cm are advocated.

For many purposes the one-vessel method is superior to the two-vessel one. Differential time lags cannot be magnified to give such large errors, but an independent measurement of the  $\text{CO}_2/\text{O}_2$  ratio is necessary if the two gases are involved. In alkaline buffers, where oxygen is the only gas, the single-vessel method is preferred. In some manometers chemicals are inserted in side tubes which maintain either the carbon dioxide or the oxygen at constant pressure. If gas equilibrium is quickly reached, these methods have the advantage of simplicity. They have been summarized by Burk, Schade, *et al.* (1951), who have proposed a three-vessel method.

A great deal of work has been devoted to the careful analysis of these manometric methods (Tonnelat, 1944) as used in measurements of photosynthesis, and Emerson and his associates have made an exhaustive study of the problem. The findings are summarized in recent books (Emerson and Nishimura, 1949; Nishimura *et al.*, 1951; Rabinowitch, 1951), and they can be mentioned only briefly here.

Emerson and Lewis (1939) obtained values for  $\Phi^{-1}$  of 8–10 photons per molecule using the two-vessel manometric method, but were able to duplicate the values of about 4 obtained by Warburg and Negelein (1922, 1923) by special adjustment of the conditions. They even obtained a  $\Phi^{-1}$  value of less than 3, which is a theoretical impossibility, indicating that the measurements were not significant. They attributed the difficulty to the Emerson carbon dioxide burst, and there is good evidence for this increase of carbon dioxide when darkened algae are first exposed to the light. Loomis (1951) obtained abnormally high pressures due to

carbon dioxide which persisted for 2 or 3 min after the light was turned off, and then decreased to a minimum before rising again to give a steady evolution of oxygen from photosynthesis. McAlister (1937), using an infrared absorption method that is specific for carbon dioxide, found that carbon dioxide continues to be given off from an algal suspension just after the light is turned on.

The manometric method calls for very careful control and impartial treatment of data, particularly in view of the fact that the total manometer-reading change is small and that the experimental error of reading the manometer, perhaps 0.5 mm, introduces a large error into the calculated value of the quantum yield. Kok (1948) gives a graph in his Fig. 20 showing many determinations of the photons per molecule  $\Phi^{-1}$  from manometric measurements, and it is clear that there is a large spread in the values.

Inouye (1951) carried out careful experiments with a two-vessel manometer duplicating, as closely as possible, the conditions of Warburg *et al.* (1950). The same apparatus and the same conditions of culturing and algal treatment were used. The results were very much like those obtained by these workers, and it was possible to get some  $\Phi^{-1}$  values of  $\pm$  photons per molecule, but if all the data are used, the calculations give  $\Phi^{-1}$  values ranging from 2 to 12. If calculated in the same way, the results of the investigators whose work was being duplicated have a similar spread.

Brackett (personal communication, 1952), as well as Emerson and colleagues (Emerson and Nishimura, 1949; Nishimura *et al.*, 1951), has made a thorough study of the errors inherent in manometry. He concludes that, at 120 oscillations per minute, over 3 min may be required for equilibration. Higher shaking rates reduce the time constant, and smaller amplitudes and denser suspensions tend to increase the time required for equilibration through the liquid-gas interface. There are dead spaces at the bottom and along the walls from 0.1 to 1 mm thick where shaking is not effective. When dense cultures are illuminated from below, a substantial fraction (up to about 15 per cent of the yield) may be developed in these dead spaces from which the gases must diffuse. This introduces a second slow process of increased importance during illumination.

According to Brackett (personal communication, 1952), if the size and shape of the vessels are different, as in some of the differential Warburg methods, the stirring efficiency of the two vessels can be widely different. Hence the equilibrium time for the small vessel may be longer than that for the large vessel, and the ratio  $\gamma$  disturbed.

Values obtained during the period of transient equilibrium show spuriously high values at the beginning, then decay logarithmically toward the correct value.

## 6. BIOLOGICAL FACTORS

One of the greatest uncertainties in the determination of the quantum yield in photosynthesis lies in the correction for respiration. Living plants and algal cells take in oxygen and release carbon dioxide in the process of respiration. This respiration goes on in the light as well as in the dark, and in most of the past researches the rate of photosynthesis has been obtained by adding the oxygen taken up in respiration to the observed net evolution of oxygen. Usually the oxygen taken up in respiration is determined by measuring the oxygen consumption while the plants or algae are in the dark. It has usually been assumed that the respiration thus determined in the dark is the same as the respiration going on in the presence of the light, and practically all quantum yields in photosynthesis have been calculated on this basis.

Kok (1951a) has reported evidence indicating that the respiration goes considerably faster in the presence of light than in the dark. Below the compensation point, where at low light intensities respiration exceeds photosynthesis, he has found that the apparent quantum requirement is smaller than it is above the compensation point, where photosynthesis exceeds respiration. Thus, quantum requirements of 8 photons per molecule were found above the compensation point. He explains his observations by assuming that the respiration below the compensation point is considerably less and the over-all apparent photosynthesis correspondingly greater. Calvin (1949) also has evidence that respiration is increased by the introduction of light.

On the other hand, Brown *et al.* (1952) carried out significant experiments with isotopic oxygen,  $O^{18}$ , using a mass spectrometer for analysis of the gases in equilibrium with a rapidly stirred suspension of algae. The decrease in concentration of  $O^{18}$  is a measure of the oxygen taken up in respiration, and this decrease goes on in both dark and light. The oxygen released in photosynthesis is released from the water and is not isotopically labeled with  $O^{18}$ . They found that in some types of algae the respiration rate is greater in the light than in the dark, but that in *Chlorella* it is the same. The evidence concerning *Chlorella*, then, is conflicting, and it is not yet known whether it is safe to assume that the respiration rate obtained under a specified set of conditions in the dark is maintained in the light. However, Brown's method seems more reliable. Further experiments and new approaches to the problem are needed.\*

Most of the measurements on photosynthetic efficiencies have been carried out with algae, and, of these, the large majority have been *Chlorella*. Three or four other types of algae, such as *Scenedesmus*, have been used, and they give about the same values as the *Chlorella*. Even salt-water diatoms (*Nitzschia closterium*) give 8-10 photons per molecule. Some

\* Added in proof: See Brackett *et al.* (1953).

experiments have been carried out with leaves of land plants placed in the Warburg manometers. A limited number of experiments have been carried out on land plants, beans, small elm trees, and even a young pine tree. Within the limits of accuracy of experiment, the energy efficiencies in photosynthesis are all about the same. This fact indicates that the process of photosynthesis under investigation is a fundamental process and is not a phenomenon connected with any one type of organism. The experiments with algae and with water plants have the advantage that the temperature control is simpler because the surrounding water is thermostated. But these experiments are handicapped by the fact that the carbon dioxide and oxygen from the organism have to obtain equilibrium with a water phase before they can be measured in the gas phase.

The algal cells are relatively simple morphological plant units in which the whole process of photosynthesis and the later reaction products are all enclosed together. In larger plants it is possible to store the chemicals produced by photosynthesis in remote parts of the plant, and this fact can alter the interpretations of the over-all photosynthetic process.

The purpose of the experiments has been to try to find the maximum efficiency in photosynthesis, and this calls for the optimum biological conditions favorable to photosynthesis. The conditions of culturing algae are rather special, but they have been easily duplicated in the different laboratories. Algae go through the normal logarithmic growth and aging that are common to all colonies of living cells. Usually the culture is used in photosynthetic experiments after the algae have been growing in a suspension for a few days or a week. After an algal suspension is more than 2 weeks old, the photosynthetic efficiency drops markedly. Obviously, dead cells or inactive cells will reduce the efficiency, because they absorb light without evolving any oxygen. There is a good deal of evidence to show that the best results are obtained with algae that are very green, with a high chlorophyll content. Several special conditions for growth of the algae have been proposed by different workers. They include specifications regarding intensity of the light on successive days, the temperature and the concentration of carbon dioxide in the gases that are bubbled through the algal suspension, and the rate of bubbling. These conditions have been duplicated in many laboratories, and algae of different strains have been traded among different workers.

Clearly it is necessary to have an ample supply of all the necessary chemicals for proper growth. The different nutrient solutions developed by Warburg, by Emerson, and by Stauffer have been described in the literature. In order to supply all the necessary chemical elements, the Wisconsin investigators used salts from sea water, soil extracts, and an A-to-Z solution containing 28 different chemical elements. It is generally recognized that a city water supply may contain chlorine or other added chemicals that are detrimental to growth, and water purified by

distillation in pyrex and in tin was used. Many of the nutrient solutions in different laboratories have been made from well water; and lake water has been tried. Rieke (1939, 1949) and others reported that quantum requirements in photosynthesis are affected by traces of certain chemical elements. Emerson and Lewis (1941, 1942) studied this problem of the trace elements and reported that the trace elements can be factors in the carbon dioxide burst. The excellent agreement between workers in many different laboratories seems to show that the ordinary methods of culturing now in use give about the same results and that trace elements needed for maximum photosynthesis are usually present.

Suggestions have been made that centrifuging of the algal cells in the dark or in the light, methods of washing the algal cells, and the sequence of exposure to bright and dim light are all factors in developing algae that will have an optimum photosynthetic efficiency. The conditions set up in one laboratory can, however, be reproduced in another laboratory. There seems to be no proof that the type of algae or the conditions of culture and growth constitute an important factor in the controversy between the groups of laboratories which get high efficiencies and those which get low efficiencies.

Information concerning photosynthesis can be obtained from measurements of the photosynthetic efficiency in bacteria (Van Neil, 1941) and from the measurements of oxygen liberation in certain chemical solutions in which nonliving chloroplast material was illuminated. French and Rabideau (1945) obtained values of 12-78 photons per molecule of oxygen released, and Ehrmantraut and Rabinowitch (1952) obtained values from 9 to 15, averaging 12. These authors consider that the quantum requirement of 10-12 for this chloroplast reaction as well as for photosynthesis represents the true measure of the efficiency of the common primary photochemical process.

It is interesting that the rate of respiration can be profoundly affected by the addition of certain chemicals such as sucrose, but when proper corrections are made for respiration, the rate of photosynthesis is unchanged. The over-all energy requirements for photosynthesis may be reduced by adding to the algae organic compounds that can be more easily reduced to carbohydrates than carbon dioxide, but attempts to find such chemicals have been unsuccessful thus far. It is clear that such an added chemical would have to penetrate the cell and be incorporated in the system of photosynthetic intermediates.

## 7. PRACTICAL APPLICATIONS

A maximum continuing photosynthetic efficiency of 35 per cent, corresponding to 8 photons of red light per molecule, can be obtained under laboratory conditions. It is interesting to compare this efficiency with

the efficiency obtained in ordinary agricultural practice. In the United States a square foot of land receives on the average about 1 kcal of solar radiation for about 500 min each day. Multiplying this total of 500 kcal/day by 43,560 (the number of square feet in an acre), we find that about 21,000,000 kcal of heat falls on an acre of land each day. About half of this radiation is in the infrared and, since it is not absorbed by chlorophyll, cannot take part in photosynthesis. Inasmuch as the efficiency of the light that is absorbed is about 35 per cent, or  $\frac{1}{3}$ , it may be concluded that only  $\frac{1}{2}$  of  $\frac{1}{3}$  of the 21,000,000 kcal can be stored in growing plants under optimum conditions. This calculation gives 3,500,000 kcal/day/acre.

This theoretical value is high for several reasons. The average corn crop of the United States in 1946 was about 35 bu/acre, which is an equivalent of about 2 tons of organic material—1 ton for the corn kernels and another ton for the cobs, leaves, and stalks. Hybrid corn on rich, fertilized land with good growing conditions can give 100 bu/acre, with a weight of 5 or 6 tons of dry organic material. A good silage crop in Wisconsin gives about  $2\frac{1}{2}$  tons/acre/year of dry organic material. Wheat and hay give of the order of 1 ton/acre/year. An aspen forest in northern Wisconsin could give 2 tons of organic material per year if it were scientifically forested and if all organic material were collected. Algae in some Wisconsin lakes produce organic material at the rate of about 2 tons/acre/year. Sugar cane growing in Hawaii the year round can give up to 40 tons of dry organic material per year.

When organic material such as sugar or wood is burned in air, it gives off about 3,500,000 kcal/ton. We have just seen that 3,500,000 kcal (corresponding to 1 ton of organic material) would be a reasonable value for the storage of an acre of sunlight through photosynthesis for 1 day, as calculated on the basis of algae grown under optimum laboratory conditions. It must be emphasized that this high value is calculated on the basis of laboratory measurements of photosynthesis over short time intervals. On this *theoretical* basis, then, the equivalent of a ton of dry plant material could be produced on an acre of land in a day, and this ton would release 3,500,000 kcal when burned in the air. There are several reasons, of course, why this extraordinary efficiency cannot even be approached in agriculture. These optimum yields imply an ample supply of water, fertilizers, and all inorganic and organic material necessary for full nutrition. The temperature must be kept at an optimum; the carbon dioxide concentration must be about 3 per cent instead of the 0.03 per cent found in ordinary air. The young plants do not cover the whole area of the ground, and only late in the growing season does all sunlight of the acre fall on the growing plants. Most important of all, the high efficiencies of photosynthesis are obtained only in light of low intensity, less than 50,000 ergs/sec/cm<sup>2</sup>, whereas the energy of bright

sunlight is of the order of 500,000 ergs/sec/cm<sup>2</sup>. In bright light the secondary thermal reaction cannot keep up with the primary photosynthetic process, and there is photooxidation of the partially reduced organic intermediates that are first formed by the photoreduction of carbon dioxide. Many more than 8 photons of light are required for the complete reduction of a molecule of carbon dioxide in intense light, because some of the photons undo the photosynthetic work that is done by the other photons. It is partly for this reason that the long summer days in the subarctic regions produce luxuriant plant growth even though the intensity of sunlight per *hour* is much less than that in the tropical areas. It is clear that 1 ton of dry plant material per acre per *day*, as calculated on the basis of optimum laboratory conditions, cannot begin to be realized in ordinary agriculture.

Instead of 1 ton of organic material per *day*, we have seen in an earlier paragraph that a good agricultural yield is only about 2 tons/acre/year. In connection with this apparently "low" yield of 2 tons/acre/year, it must be remembered that the growing season is only about 100 days long, whereas the calculations for the annual solar energy involve 365 days, the majority of which are not suitable for agriculture in the United States because of the winter temperatures. The agricultural crop of 2 tons/acre per growing-year of 100 days is of the order of 2 per cent of what the growth might be (1 ton/day for 100 days) if the conditions were as favorable as they are in the laboratory experiments with algae.

This 2 per cent efficiency represents *agricultural* efficiency. If the efficiency of energy storage is calculated on the basis of total annual radiation, including infrared as well as visible light and the winter season as well as the summer season, the agricultural crop of 2 tons/year corresponds to a storage of only

$$\frac{2 \times 3.5 \times 10^6 \text{ kcal}}{365 \times 21 \times 10^6 \text{ kcal}}$$

or about 0.001. The 2 tons of organic material produced in a year would thus give back through combustion, at  $3.5 \times 10^6$  kcal/ton, only 0.1 per cent of the total annual solar radiation.

Although improvements in agriculture have greatly increased the utilization of solar energy, it will be difficult to make improvements great enough to approach the theoretical limit calculated here. In the last few years considerable interest has developed in the possibility of growing algae in tank farms, which might achieve greater production of organic material per acre than is now possible under ordinary methods of agriculture. The capital investment for metal or concrete tanks or for glass enclosures to contain the algae and provide means for increasing the carbon dioxide content of the air would seem to be prohibitive from an economical standpoint. However, some attention has been given to the use

of cheap plastic bags for containing algal suspensions. There are many problems to be solved, such as the filtering of algae, the increased supply of carbon dioxide, and the temperature control, but interesting explorations have been begun (Burlaw *et al.*, 1952; Geoghegan, 1951; Kok, 1951b; Myers *et al.*, 1951; and Tamiya, 1949).

One of the interesting challenges is to devise means by which the algae can be exposed to the light for a fraction of a second and then given a rest period in the dark under conditions such that the whole area can still be exposed continuously to the sunlight. If dark resting periods can be economically arranged, it may be possible to grow the algae at a very high light intensity with a higher efficiency than is now possible. Investigations might well be undertaken also in the hope of breeding algae or plant material that will be able to carry out photosynthesis with the high 30 per cent efficiency even in bright sunlight. Although algae are particularly suitable for tank farming, they do not have the mechanisms that exist in higher plants for storing plant products, and it may be that a higher plant of lower photosynthetic efficiency than algae may be more suitable than algae for utilizing large fractions of the solar energy when the light is as intense as full sunlight.

## 8. DISCUSSION

A brief survey of the investigations of energy efficiency in photosynthesis to mid-1952 is given in Table 4-1. Full details of the work may be found in the references given. Warburg and his associates have maintained for thirty years that the energy required is about 4 photons per molecule, the light being utilized with an efficiency of about 70 per cent. More recently, they claim an efficiency up to 92 per cent. Emerson, Duggar, Stauffer, Daniels, Rabinowitch, Arnold, and others have maintained for many years that the normal value is about 8-10 photons per molecule, with a light utilization of about 25 per cent. Rieke first obtained 4 but later considered 8 photons per molecule to be the normal value. Franck has tried to explain the discrepancy, and he has been associated with the 8-photon group.

The discussion and critical evaluation of these different researches has been greatly simplified by an excellent monograph by Rabinowitch (1951) which deals with all the work very thoroughly. Rabinowitch shows clearly the possible errors in interpretation of manometry which can lead to such widely different conclusions from the laboratories of the ablest scientists. Emerson and Nishimura (1949) and Rieke (1949) cover the situation to 1949, and Nishimura *et al.* (1951) to mid-1951. The manometric measurements would be simple if only straightforward chemical reactions were involved, but the reverse reaction of respiration, the holdup of intermediate compounds in the process of photosynthesis, and

TABLE 4-1. CHRONOLOGICAL REVIEW OF INVESTIGATIONS ON ENERGY EFFICIENCY IN PHOTOSYNTHESIS

Year	Investigators	$\Phi^{-1}$ (photons per molecule)	Experimental conditions <sup>a</sup>
1922, 1923	Warburg and Negelein	4	Single-vessel manometer; complete absorption of light; respiration greater than photosynthesis; 1000 ergs/sec/cm <sup>2</sup>
1929	Briggs	~10	Gas analysis; bean and elm; 5000 ergs/sec/cm <sup>2</sup>
1934	Duggar <i>et al.</i>	~16	Gas analysis
1935	Gabrielsen	~10	Gas analysis; plant leaves
1938	Manning, Stauffer, <i>et al.</i>	~20	Gas analysis on CO <sub>2</sub> and O <sub>2</sub> ; chemical titration for O <sub>2</sub> ; 800-24,000 ergs/sec/cm <sup>2</sup>
1938	Manning, Juday, and Wolf	~20	Chemical titration for O <sub>2</sub> ; algae in bottles submerged in lake
1938	Manning	.....	Review article
1938	Burns	~9	Gas analysis; 2 hr illumination; pine tree; 10,000 ergs/sec/cm <sup>2</sup>
1939	Emerson and Lewis	~8-10	Two-vessel manometer; accurate measurements; analysis of manometric errors; discovery of CO <sub>2</sub> burst; impossible apparent value of $\Phi^{-1} = 3$ obtained by taking advantage of burst
1939	Rieke	4-5.5	Manometer; pretreatment of algae; time lags noted
1939	Eichhoff	4-5	Manometer; carbonate buffer
1939	Petering <i>et al.</i>	10-16	Dropping-mercury electrode for oxygen; quick response in measurements; less than half of light absorbed
1939	Stauffer	10-15	Manometer, using same algae and experimental conditions as those of Petering, Duggar, and Daniels (1939)
1939	Magee <i>et al.</i>	13 (9-20)	Microcalorimeter; light absorbed and heat evolved measured
1939	A.A.A.S.	.....	Symposium on photosynthesis
1940	A.A.A.S.	.....	Symposium on photosynthesis
1941	Emerson and Lewis	9-1	Manometer; carbonate and acid buffers same; trace elements necessary; several different types of algae used
1941	Dutton and Manning	10	Dropping-mercury electrode; marine diatoms <i>Nitzschia closterium</i> ; $\Phi^{-1}$ same at 6650-4047 Å; other pigments than chlorophyll utilized in photosynthesis
1941	Franck and Gaffron	11	Review article

1942	Emerson and Lewis			Manometer; role of pigments other than chlorophyll
1943	Emerson and Lewis		8	Manometer; $\Phi^{-1}$ at different wave lengths; constant at 5800-7300 Å; minimum at 4850 Å
1944	Tonnelat		12-(78)	Microcalorimeter; 16 hr illumination; 5000 ergs/sec/cm <sup>2</sup>
1945	French and Rabideau		16	Chloroplasts <i>in vitro</i> ; Hill reaction by manometer; 1400-8000 ergs/sec/cm <sup>2</sup>
1946	Wassink and Kersten		4	Manometer; $\Phi^{-1}$ same in red, yellow, and green; <i>Chlorella</i> and <i>Nitzschia</i>
1946	Wassink		8-10	Discs of various types of leaves in Warburg manometer
1946	Warburg		4	Manometer
1947	A.A.A.S.		7-8	Several papers on quantum requirement
1948	Warburg			Manometer
1948	Kok			Manometer; 4 photons per molecule below compensation point; probably explainable on basis of altered respiration
1948, 1949				Emerson invited by Warburg to work in his laboratory at the University of Illinois; disagreement in interpretation of results not resolved
1949	Emerson and Nishimura		10	Manometer; criticized Warburg's results, considered that CO <sub>2</sub> burst could be missed
1949	Moore and Duggar		9-12	Dropping-mercury electrode for oxygen; quick response, consistent results; $\Phi^{-1}$ calculated from increase in intensity of light with two light beams as well as light and dark
1949	Rieke		9-11	Manometer; integrating sphere to catch all scattered light; alkaline buffer with O <sub>2</sub> alone, and acid buffer with CO <sub>2</sub> and O <sub>2</sub> ; <i>Chlorella</i> and <i>Scenedesmus</i> ; 1900-4000 ergs/sec/cm <sup>2</sup> ; reactions with hydrogen
1949	Arnold		9-12-20	Microcalorimeter; <i>Chlorella</i> and <i>Scenedesmus</i>
1949	Burk <i>et al.</i>			Manometer; ethyl chlorophyllide actinometer; increased light rather than light and dark
1949	Warburg <i>et al.</i>			Manometer; totally absorbed red light does not inhibit respiration
1950	Warburg and Burk		10 with carbonate buffer of pH 9; 2.5-4.8 with acid culture medium	Manometer; cultural conditions described; deep well water used
1950	Went		~15	Tomato plants grown under optimum conditions; energy efficiency estimated from dry weight of plants

TABLE 4-1. CHRONOLOGICAL REVIEW OF INVESTIGATIONS ON ENERGY EFFICIENCY IN PHOTOSYNTHESIS.—(Continued)

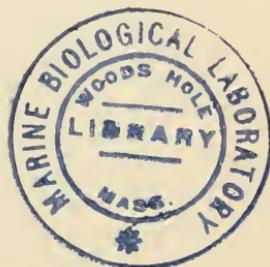
Year	Investigators	$\Phi^{-1}$ (photons per molecule)	Experimental conditions <sup>a</sup>
1951	Rabinowitch	.....	Complete history and critical analysis of experimental measurements of energy efficiency in photosynthesis
1951	Warburg and Burk	(1.2)–4.6	Manometer; exposures 1 min and longer; $\Phi^{-1}$ decreased with length of exposure
1951	Warburg and Geleick	3.0 for CO <sub>2</sub> ; 3.5 for O <sub>2</sub>	Manometer; light compensating respiration, plus intermittent light, up to 9 hr; 88% of light utilized
1951	Warburg, Geleick, and Briese	2.8–2.9 in acid medium; 9.0 in 25% carbonate; 4.6 in 10% carbonate	Manometer, two-vessel method; manometer, one-vessel method
1951	Warburg	(1) <sup>b</sup>	Manometer; outer vessel containing ethyl chlorophyllide, actinometer surrounding inner manometer vessel; 3-min intervals 1 min light and 1 min dark; light of high intensity
1951	Burk, Cornfield, and Schwartz	(1) <sup>b</sup>	“Emerson burst” of CO <sub>2</sub> found with algae cultured like those of Warburg <i>et al.</i>
1951	Loomis	.....	Review and critical survey
1951	Franck	9	5200–6800 Å; light absorbed by fucoxanthol was photoactive
1951	Tanada	12	Photochemical production of oxygen from chemicals sensitized with chloroplasts; related to photosynthesis
1951	Ehrmantraut and Rabinowitch	(2–15)	Manometer, close duplication of experimental conditions of Warburg and Burk (1950), wide variations in $\Phi^{-1}$ and no conclusive value
1951	Inouye	(2–15)	Manometer, close duplication of experimental conditions of Warburg and Burk (1950), wide variations in $\Phi^{-1}$ and no conclusive value
1951	Nishimura <i>et al.</i>	10	Manometer; carbonate buffer, criticism of Warburg's two-vessel experiments
1951a	Kok	(8 above compensation; 4 below compensation)	Respiration slower in dark than in light, leading to lower apparent quantum requirement below compensation

1951	Warburg, Burk, and Schade	3-5	Manometer; time lag in carbonate buffers; transmission actinometer, thin suspension of algal cells
1951	Burk, Schade, <i>et al.</i>	4	Manometer, two-vessel and three-vessel methods; red, yellow, and green; above and below compensation; total or partial absorption of light; side arms for gas-removing agents
1951	Evans	9-10	Direct measurements of O <sub>2</sub> by magnetic moment and CO <sub>2</sub> by infrared absorption; analysis of scattering of light and other possible errors
1952	Warburg <i>et al.</i>	3.5	Manometer; single vessel; 5% carbonate; 1-min sequence, dark and light; thin suspension; transmission actinometer
		3.9, 3.8, 3.7, 4.2 for 1st, 2nd, 3rd, 4th hr	Washing cells affects $\Phi^{-1}$
1952	Brown <i>et al.</i>	.....	Respiration of <i>Chlorella</i> unaffected by light as determined by mass-spectrometric respiration analysis with O <sub>18</sub> ; some other organisms were affected by light
1952	Schwartz	6-8	Dropping-mercury electrode; green light; culture conditions duplicating those of Warburg and Burk
1952	Whittingham	.....	Review of the literature
1953	Braekett	8.5 (6-13.5)	Oxygen by platinum electrode and special equipment; very quick response; $\Phi^{-1} = 8.5$ average; in a few cases $\Phi^{-1} = 6$ ; chlorophyll content important
*1954	Yuan <i>et al.</i>	8.9 $\pm$ 1.0	CO <sub>2</sub> and O <sub>2</sub> recorded simultaneously: CO <sub>2</sub> by infrared, O <sub>2</sub> by magnetic moment

<sup>a</sup> All measurements are on algae, usually *Chlorella*, unless otherwise specified. All manometric measurements made use of the two-vessel technique unless otherwise specified.

<sup>b</sup> First photoprocess.

\* Added in proof.



the time lag in the manometers and in the biological reactions lead to a very complicated situation that can give different rates of change in the gas pressure depending on the conditions prevailing in the manometers and on the pretreatment of the algae. Starting in 1939 with the investigation of Emerson and Lewis and continuing to the present time, Emerson and his associates have obtained consistently a quantum requirement of 8–10 photons per molecule, and they conclude that in Warburg and Burk's experiments, which indicated a requirement of about 4 photons per molecule, insufficient allowance was made for the time lag, and the differential time lag in particular. These time lags may make large errors in the two-vessel manometric method. The manometric measurements of Emerson and Lewis gave results of exceptionally high precision.

There is no controversy among those who determine the energy efficiency by nonmanometric methods. These methods always lead to quantum-requirement  $\Phi^{-1}$  values of about 8 photons per molecule, with occasional values somewhat less. With the exception of the work of Warburg and Burk and their associates, most of the other workers using the manometric method have obtained values in agreement with those obtained by the nonmanometric methods.

Petering *et al.* (1939) used a polarigraphic method specific for oxygen and obtained values of about 10 photons per molecule. Stauffer (1939) used a two-vessel manometric method at the same time with the same algae, the same cultural techniques, and the same light standardization. He too obtained independent values of about 10 photons per molecule. Rabinowitch (1951) concludes that "the quantum requirement of  $10 \pm 2$  represents the true measure of the efficiency of the common primary photochemical process. The quantum requirements of much less than 8 reported by Warburg and Burk in acid media are the only ones which do not fit into this picture. Whether this discrepancy is caused by a systematic experimental error, as suggested by Emerson and coworkers, or to the substitution for true photosynthesis of a partial reversal of respiration requiring a smaller number of quanta is an independent and controversial question."

After further perfecting of the polarigraphic method for oxygen, Moore and Duggar (1949) obtained very consistent results, as shown in Fig. 4-1. It is to be noted that in this method, which measures only the dissolved oxygen (without the complication of carbon dioxide and the transfer of gases into the gas phase), quantum requirements of 9–11 photons per molecule were obtained under conditions where respiration exceeded photosynthesis and where photosynthesis exceeded respiration. Furthermore the same value was obtained when changing from dark to light or when changing from a given light intensity to a higher light intensity. Within the limits of accuracy of the experiments, the results were the same for red, blue, or green light.

Several important investigations have been carried out in 1951 and 1952, subsequent to Rabinowitch's book (1951) and to Emerson's researches (Emerson and Nishimura, 1949; Nishimura *et al.*, 1951) on the manometric difficulties.

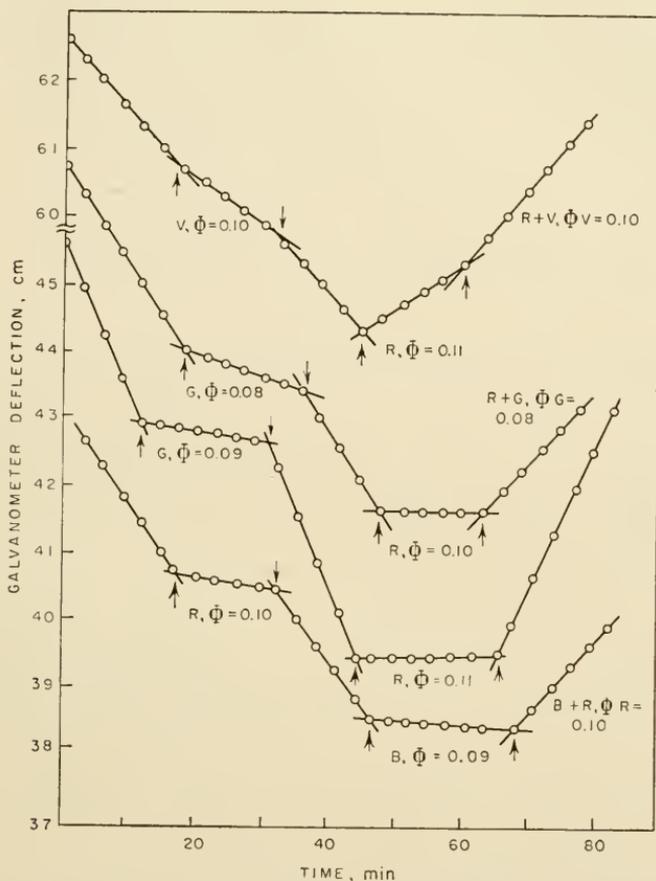


FIG. 4-1. Changes in the concentration of dissolved oxygen with time in algal-cell suspensions in light and in darkness. The light was turned on at the point indicated by the arrow pointing upward, and off at the point indicated by the arrow pointing downward. The quantum efficiencies, in molecules per photon, are indicated by  $\Phi$ . The quantum requirement would be  $1/\Phi$ . V = violet; R = red; G = green; B = blue.

Brackett (Olson *et al.*, 1949) has developed a method for measuring dissolved oxygen quickly and accurately, using a special platinum electrode and an alternating current. He can determine the concentration of dissolved oxygen in a suspension of algae in less than 10 sec. With this apparatus Brackett (personal communication, 1952)\* has carried out a very careful investigation on the time lags in photosynthesis under a

\* Added in proof; Brackett *et al.*, 1953a.

variety of conditions. The quantum requirements of photosynthesis as determined by him and his associates show considerable variation, with a median of 8.5 photons per molecule. Plotted against chlorophyll concentration, the yields fall in a band about 2 quantum units wide, the logarithm of the yield increasing with chlorophyll concentration and extrapolating to limits of 6 and 8 photons per molecule for the highest concentration. Emerson and Lewis (1939) had also found that the efficiency increased with chlorophyll content—thus the greener the algae, the more efficient the storage of energy. Chlorophyll density and high respiration are probably both indicative of healthy algal cells, which should give normal photosynthesis.

In a study of the dependence of quantum yield on light intensity, Brackett (personal communication)\* found that the evaluation of the respiratory contribution played an important role. Using average dark values of respiration, he confirmed the findings of Kok (1951a) that high apparent efficiencies of 4 photons per molecule or less appeared below the compensation point. However, marked increases in respiration were found following illumination. For a given culture, respiration can be reduced to a very low rate by dark adaptation or raised to a high rate by light adaptation without any effect on the quantum efficiency. If light-respiration values determined by interpolation are used, the photon requirements and light efficiency become independent of light intensity. Thus the 4 photons per molecule below the compensation point can become 8, as suggested by Kok, if proper correction is made for respiration. Brackett believes that this may explain many of the observations of higher efficiency at low light intensities. The photon requirement ranged from 6 to 13.5 molecules per photon, depending on the intensity of light, the time factor, and the previous history of the algae both in their cultural conditions and in their immediately preceding exposure to light.

Brackett concludes that, after dark adaptation, the initial rate of photosynthesis is very small and that it increases to a constant value in 1–3 min. The induction effect decreases after several alternations of dark and light, and this adaptation appears to carry over several minutes of darkness.

Kok (1951a, 1951b) has carried out an extensive set of experiments and concludes that the minimum quantum requirement is 7–8 photons per molecule, but that at low light intensities, where respiration exceeds photosynthesis, the changes in respiration lead to an apparent quantum requirement of 4 photons per molecule. He reports a definite change in the quantum requirement at the compensation point.

Warburg and Burk and their associates have published many papers in the period 1950–1952 and have made improvements in apparatus and

\* Added in proof: Brackett *et al.*, 1953b.

changes in techniques. They continue to report 4 photons and sometimes even 3 photons per molecule. Most of their recent work has been done with the excellent ethyl chlorophyllide actinometer, which simplifies the experiments and gives a reliable check on the absolute measurements of the light intensity. Using a transmission actinometer in the form of a vessel of ethyl chlorophyllide solution surrounding the reaction cell, they have been able to use thinner algal suspensions that transmit some of the light. These conditions are much better than those of their earlier experiments in which thick algal suspensions were used so as to absorb all the light. In the thick suspensions the conditions of exposure range all the way from full light intensity to complete darkness, and the shaking gives rise to the complication of unknown, intermittent exposures to dark and light. Moreover, in the thick suspensions, respiration overbalances photosynthesis.

Another change involves the use of a constant, unmeasured light exposure to offset respiration and the superposition of monochromatic light of measured intensity. This use of two light beams was tried also by Moore and Duggar (1949).

In this way the extra molecules of oxygen evolved per photon of extra light absorbed permit a calculation of the quantum yield. The intensity of the background light and the quantum requirement in this light are unknown, and it must be assumed that they fall in the region of linearity on the Blackman curve. In the dark-light experiments it must be assumed that respiration continues in the light at the same rate as in the dark. In the light-brighter light experiments it must be assumed that respiration and the quantum requirement of photosynthesis both remain constant.

A new development in the laboratories of Warburg and of Burk is the reporting of evidence for a 1-quantum process. Certainly, everyone would agree that the primary photoactivation process must be a 1-photon process, but the time lags following changes from dark to light, as already described, are such that they would seem to make difficult the determination of significant quantum-requirement measurements in periods of 1 min dark and 1 min light, such as have been used in these experiments in which a 1-quantum process has been reported. As yet, no 1-quantum results have been reported by Warburg or Burk in alkaline solutions, nor have there been checks to date by other laboratories in acid solutions. They are, of course, thermodynamically impossible as a continuing process.

Evans (1951) has determined both oxygen and carbon dioxide with methods that are specific for the two gases. He found a quantum requirement of 9-14 photons per molecule, as calculated for oxygen and independently and simultaneously for carbon dioxide. The experimental

operations are difficult, and the investigation is being continued with the help of recording instruments.\*

Any method that will give correct and unambiguous analyses for the concentrations of both carbon dioxide and oxygen simultaneously and quickly under a wide variety of light intensities will go a long way toward straightening out some of the differences in manometric results and helping to understand the mechanism of photosynthesis.

Schwartz (1952) tried to repeat with the dropping-mercury electrode the high efficiencies of 3-4 photons per molecule which he had obtained in Burk's laboratory. Using the same methods of algal culture and treatment, he obtained values of 5-8 photons per molecule, with most of the values centering around 8.

It is extremely significant that the reported high efficiencies of 4 photons per molecule have been obtained only with the manometric method. Some workers urge that the manometric methods, which are subject to these uncertainties, should receive less attention and that greater effort should be devoted to the development and use of methods that give quantitatively and specifically the concentrations of oxygen, carbon dioxide, and carbohydrate. Other workers, however, contend that, inasmuch as the 4-photon results have all been obtained with manometers, it is necessary to continue the manometric studies in order to show what errors of interpretation may have been made and thus to establish the "about 8" photon value for the manometric methods to bring them into agreement with the nonmanometric values of about 8.

The type of algae, the conditions of culture, and the absolute measurements of energy do not seem to be factors in the disagreement, because they have been so thoroughly checked. An earlier suspicion that the calibrating standard for light measurements might be different has been dispelled. All measurements are now referred back eventually to the U.S. Bureau of Standards standard carbon-filament lamp. Moreover, they have been cross-checked with Warburg's ethyl chlorophyllide actinometer.

Throughout the whole controversy the constancy of respiration looms large as an uncertainty. In most of the calculations it is assumed that respiration goes on at the same rate in the light as in the dark. If this is not the case, serious errors can be introduced. The matter is under investigation along several different lines.

Still more research is necessary to emphasize the nature of erroneous conclusions based on the transient conditions which follow light-dark and dark-light transitions. Transient chemical changes may profoundly affect gas exchange measurements over short time intervals.

\* Added in proof: Yuan (1954, 1955) obtained specific records of carbon dioxide and oxygen simultaneously. Forty measurements of  $\text{CO}_2$  averaged  $8.7 \pm 1.0$  photons per molecule while 31 measurements of  $\text{O}_2$  averaged  $9.1 \pm 1.2$  photons per molecule.

Reducing to an oversimplification, the 8-photon advocates believe that the 4-photon results are in error because of misinterpretations of the manometric data involving both time lags and physiological complications, and the 4-photon advocates believe that the 8-photon workers are not using their algae under the most favorable conditions. However, the 8-photon workers have used the same cultures and conditions as the 4-photon workers and have consistently obtained the 8-photon values over periods of many years.

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## CHAPTER 5

# The Mechanism of Photosynthesis

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*Introduction. General characterization of the photosynthetic process. The photosynthetic apparatus and preliminary remarks on energy transfer. Evidence for the mechanism of photosynthesis as outlined in Sect. 2: Observations and considerations of a comparative biochemical nature—The combined study of photosynthesis and chlorophyll fluorescence—Tracer experiments—Experiments on isolated chloroplasts—The connection between redox potentials and photosynthesis—On the connection between phosphate metabolism and photosynthesis. Some remarks on energy transfer in photosynthesis. Summary. References. Addendum.*

### 1. INTRODUCTION

In older literature the process now called "photosynthesis" was often simply denoted as "assimilation" or, more precisely, as "carbon dioxide assimilation." The inadequacy of these older terms has gradually been recognized. "Assimilation" should mean all kinds of conversion of substrates into cell constituents, including reserve substances; "carbon dioxide assimilation" should indicate all cases in which carbon dioxide is incorporated into these compounds. In this sense both terms cover a range of phenomena wider than photosynthesis. The term "photosynthesis" is now restricted to the conversion of carbon dioxide into cell constituents, chiefly carbohydrates, by the interaction of radiant energy. The discrimination between carbon dioxide assimilation and photosynthesis became desirable with the recognition—about 1935—that various types of heterotrophic cells more or less continually incorporate carbon dioxide into metabolic products (cf. Klyver, 1939). After 1940 this view was corroborated by tracer studies (Van Niel *et al.*, 1942). Moreover, the chemosynthetic carbon dioxide assimilation of certain bacteria capable of oxidizing inorganic substrates, yielding, for example, nitrates and sulfates, has been known as a counterpart of photosynthesis for more than sixty years.

These considerations suggest that the assimilation of carbon dioxide by way of photosynthesis is not completely isolated in a biochemical sense and that light is not strictly necessary for the incorporation of

carbon dioxide into cell constituents. The question as to the role of the light energy is thus specified. Indeed, various lines of research to be discussed later lead to the concept that carbon dioxide is not directly concerned with the action of light.

The connections that have been recognized, however, between photosynthesis and other biochemical processes of carbon dioxide incorporation have decreased neither the importance of the photosynthetic process in nature nor its unique position in photobiology. It is well known that photosynthesis constitutes the only path by which organized nature has access to the solar energy. In fact, a very high percentage of the energy mankind uses has its origin, directly or indirectly, in photosynthesis of present or past time. On the other hand, even the most efficient crops fix hardly more than 2 per cent of the photosynthetically utilizable solar radiation falling on the cultivated area (Wassink, 1948a). Scientists have recently turned attention to this situation and have raised the question as to which limiting factors are operative in decreasing the efficiency of the sunlight, since in direct laboratory measurements photosynthesis shows a yield as great as 20-30 per cent. Cultivation of unicellular algae has been suggested as possibly giving a larger gain of solar energy. Higher yields might conceivably be obtained, since in relatively thick, stirred suspensions of these organisms the incident radiation could be more evenly distributed among the whole of assimilating tissue than in a land plant, and an optimal carbon dioxide content might be easier to maintain. These organisms, moreover, are usually more flexible in their metabolism than higher plants are, so that it seems possible to cultivate one and the same organism as carbohydrate or fat plant, depending on the culture conditions. Important work in this respect has been done especially by Spoehr and Milner (1949). In the laboratory of the present writer, work on mass culturing of *Chlorella* was started in connection with an examination of the basic metabolic processes and energy relations. A more comprehensive treatment of related questions is to be found in Chap. 4.

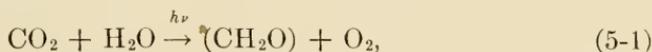
Photosynthesis is distinguished from most other photobiological processes in that light absorption leads to a gain in energy in the system. It is well to realize that in most, or possibly all, other biological processes the intervention of light is not connected with any appreciable gain in energy; i.e., light absorption initiates or directs only catabolic processes. Another distinction, which is still not fully developed, is that in photosynthesis the effect expressed in energy change never surpasses the energy of the absorbed light and mostly involves only a fraction of it, so that appreciable effects arise only after the absorption of considerable amounts of light. In many other cases, such as the various actions of ultraviolet light and phototropism, the primary effect is exerted by 1 quantum or a few quanta of light, finally leading to a reaction in which large numbers

of molecules are involved (Wassink, 1946a, 1954\*). The action of the light is that of a stimulus, in Pfeffer's sense, and in more recent times the connection between the primary action and the final effect has been suitably termed "amplification" (Jordan, 1932, 1938). Biochemically it can be visualized that in these cases an enzyme or carrier molecule is hit in an early stage of the reaction chain, thus influencing a lot of molecules moving along this path in much the same way as the grid in a radio tube directs and modifies the energy stream (Wassink, 1946a,b). In these cases the light energy plays hardly any role in the energy balance of the process. In photosynthesis, on the other hand, the light energy is essential to complete the energy balance of the process and, other things being equal, of the cell. This statement is not invalidated by the fact that some types of photosynthesis exist (e.g., in colored sulfur bacteria) in which the gross reaction mechanism requires hardly any supply of energy. Even in these cases some intermediate step also probably requires a stoichiometric intervention of light energy for its completion.

One remark should be added. In the future, it may become desirable to denote "photosynthesis" as "carbon dioxide photosynthesis" or "carbohydrate photosynthesis." It was mentioned previously that the reduction of carbon dioxide very probably takes place in the dark with the aid of something prepared in the light. Other cellular processes such as nitrate reduction or formation of energy-rich phosphate bonds might turn out in the future to have essentially the same relation to light as carbon dioxide reduction.

## 2. GENERAL CHARACTERIZATION OF THE PHOTOSYNTHETIC PROCESS

Under the influence of light energy the green plant performs the over-all reaction



in which  $(\text{CH}_2\text{O})$  stands for carbohydrate in general. Actually the process going on in the plant consists of a number of links or partial reactions, to be discussed later in greater detail. Carbon dioxide assimilation, moreover, may result in growth of the plant, which means that photosynthesis is linked with other processes such as protein formation. This will be the more so in cells such as quickly multiplying algae, which, while photosynthesizing, show an active over-all metabolism. But Smith (1943) showed that, in adult leaves of certain plants, the photosynthetic process very closely approaches the over-all reaction, Eq. (5-1).

Early in the study of photosynthesis it was recognized that only the green parts of plants are capable of carbon dioxide assimilation. Ingen-Housz (1779), in particular, clearly demonstrated the effect of light and

\* See Addendum.

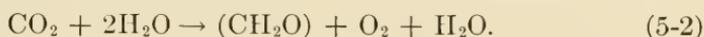
the connection of the oxygen evolution with the green parts of plants. At that time a very active study of photosynthesis was taking place, issuing chiefly from Priestley's preliminary observations on the purifying action of green plants on the surrounding air. The essential role of light in performing this effect, for the discovery of which Ingen-Housz is generally credited, was independently discovered by van Barneveld (1781), as was pointed out by Rauwenhoff (1853). A year before Ingen-Housz's well-known study (1779) appeared, van Barneveld concluded that light has a beneficial effect upon the oxygen production of plants. His observations, however, were less profound and less profuse than those of Ingen-Housz, and moreover, for various reasons, his paper appeared later than that of Ingen-Housz. Also, in popular-science treatises of those days suggestions are found that plants derive their nourishment chiefly from the air and that light is essential for their existence (cf. Wassink, in press).

The "leaf green," for which Pelletier and Caventou as early as 1818 created the name "chlorophyll," in the higher plants is contained in chloroplasts. These are by no means homogeneous but contain smaller green entities, *grana*, which, in their turn, again are complicated structures. It is very probable that chlorophyll and also other pigments related to it or associated with it are active in photosynthesis only when intimately linked to a protein bearer. This view is relatively young; it was initiated especially by Lubimenko and followed in later years by Mestre, Stoll, Baas Becking, and many others (for references, see Wassink, 1948b). Willstätter and Stoll (1918) have been making attempts to carry out the photosynthetic process *in vitro*, starting from aqueous suspensions of highly purified chlorophyll in contact with carbon dioxide. The general development of enzymology, together with these facts, would lead one now to consider these trials as pioneer attempts with little foundation in nature. Nevertheless the study of chlorophyll in solutions or in aqueous suspensions may yield very valuable information concerning certain features of the photosynthetic process, especially light absorption, energy transfer, and photosensitization (cf. Livingston, 1949). But the complete photosynthetic process so far has been found only in intact cells. Parts of the process, especially the evolution of oxygen with the simultaneous reduction of certain special hydrogen acceptors, e.g., quinone, have been realized with preparations containing chloroplasts or parts thereof.

Photosynthesis, like probably all important biological processes, is not such a simple reaction as Eq. (5-1) might suggest but is a sequence of partial processes in which the state given by the right-hand side of the equation is gradually approached. Chiefly since the work of F. F. Blackman (1905) and his collaborators, it is known that besides a specific photochemical reaction a dark reaction can be distinguished in the sequence

of the process. Each of these reactions may act as the master reaction governing the total rate of the process; the reaction that predominates depends on which environmental condition is the limiting factor. In this way properties of both reactions have been established, e.g., the quantum yield of the photochemical reaction and its independence of temperature, and the cyanide and temperature sensitivity of the dark process (cf. Warburg, 1925; Warburg and Negelein, 1923; Wassink *et al.*, 1942). It was found, furthermore, that what has been denoted as "dark reaction" consists of several processes, each of which may act as the master reaction, depending on external limitations.

It follows from Eq. (5-1) that the ratio of CO<sub>2</sub> absorbed to O<sub>2</sub> evolved theoretically equals unity. In many cases this quotient does not deviate much from 1. This suggests that the oxygen evolved originated from carbon dioxide, a conclusion later found to be unjustified. Arguments were collected for the view that carbon dioxide is reduced by hydrogen derived from the water from which the oxygen is evolved. The oxygen of carbon dioxide enters partly into the organic matter formed, partly into water. Equation (5-2) does more justice to this viewpoint than Eq. (5-1):

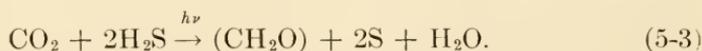


The correctness of this view was proved by investigations that made use of the isotope O<sup>18</sup>. Ruben *et al.* (1941) used either water or carbon dioxide artificially enriched in O<sup>18</sup> in photosynthesis experiments. The O<sup>18</sup> content of the evolved oxygen was always close to that of the water and was distinctly different from that of the carbon dioxide used in the experiments. It has been argued that this result probably was not fully conclusive, since, with a reasonable rate of exchange in the cells or in the chloroplasts, the excess of water would easily give rise to the observed effect. This criticism, however, does not apply to similar experiments by Dole and Jenks (1944), who made use of the natural small differences in O<sup>18</sup> between water and carbon dioxide with essentially the same result as that of Ruben *et al.* The restriction should be made that, strictly speaking, the result is valid only for the objects investigated, i.e., a green alga and a few land plants. There is, however, no reason to suppose that other plants will behave differently.

One type of facts fails to fit into this scheme. It is assumed that the oxygen of the air has originated chiefly from the photosynthetic process, or at least that the oxygen now present has passed through the metabolic cycle many times. From the above statements it would be assumed that the O<sup>18</sup> content of the atmospheric oxygen would closely resemble that of water and not that of carbon dioxide (or of carbonate rocks). Actually the reverse is true (Kamen and Barker, 1945). Notwithstanding the fact that this discrepancy has still to be clarified, it is not considered

sufficient to invalidate the more direct evidence derived from the  $O^{18}$  experiments.

The view that the photosynthetically produced oxygen originates from water was held long before isotope studies were introduced into biological research. This opinion arose from comparing the photosynthetic process of green plants with that of purple sulfur bacteria (Van Niel, 1931). These organisms, for example, realize the following over-all conversion:



A comparison of Eqs. (5-3) and (5-2) strongly suggests that in green plants the relation between water and oxygen is the same as that between hydrogen sulfide and sulfur in purple sulfur bacteria. This means that the oxygen in green-plant photosynthesis arises from a photochemical<sup>1</sup> decomposition of water.

Study of the photosynthetic process of purple sulfur bacteria (Thiorhodaceae), of the related green sulfur bacteria (Thiochloraceae), and of the nonsulfur purple bacteria (Athiorhodaceae) reveals many interesting facts, which in some respects have made these bacteria advantageous over green plants for the study of the process. The most obvious feature is that carbon dioxide is photochemically reduced by these bacteria at the expense of substances other than water.\* In green-plant photosynthesis, only water, so far, has been known to act as a hydrogen donor for the photochemical process, whereas with most of the bacteria already mentioned *various* reduced substances can act as such. Purple sulfur bacteria, for example, can reduce carbon dioxide not only with hydrogen sulfide, sulfur, sodium thiosulfate, and other reduced sulfur compounds but also with various organic substances, especially aliphatic acids, e.g., butyric acid (Muller, 1933), and with molecular hydrogen. Instead of carbon dioxide, organic carbon compounds, especially acids of a more oxidized type, e.g., malic acid, can be used as substrates for photosynthetic conversions (*ibid.*). Whereas the Thiorhodaceae in their development are obligately anaerobic and obligately photosynthetic (Roelofsen, 1935), some Athiorhodaceae can develop either anaerobically photosynthetically or aerobically heterotrophically, in each case using much the same substrates (Van Niel and Muller, 1931).

Although certain Thiorhodaceae (e.g., *Chromatium*, strain D) in fairly dense suspensions may remain alive and motile in the open air for a very long time, development from a small inoculum often fails to start when the compounds in the medium are not sufficiently reduced. The present writer, for example, occasionally observed that cultures in stoppered bottles completely filled with boiled culture medium did not grow unless a drop of a sodium sulfide solution was added (unpublished work).

<sup>1</sup> In this connection the term "photochemical" does not imply that in this process no dark-reaction steps are included.

From this discussion it is clear that, in purple bacteria, independent variation of the hydrogen donor and of carbon dioxide and allied compounds is possible, which suggests that these two "poles" of the photosynthetic chain are independent to a high degree.

Various facts to be discussed later on strengthen this view. It appears that the photosynthetic mechanism can be rendered essentially in three reaction complexes (Dorrestein *et al.*, 1942; Wassink, 1947), namely:

(1) A dark-reaction complex in which the hydrogen donor is dehydrogenated.

(2) A photochemical-reaction complex in which light energy is fixed.

(3) A dark-reaction complex in which carbon dioxide is converted into carbohydrate.

The arguments for this view are derived chiefly from the following types of studies: (1) observations of a comparative biochemical nature, (2) combined study of chlorophyll fluorescence and photosynthesis, (3) tracer work, (4) *in vitro* studies of partial reactions, (5) combined study of photosynthesis and redox potentials, and (6) studies on the relation of phosphate conversion and photosynthesis. In the following sections are discussed the chief observations of these types. This discussion will be preceded by some remarks on photosynthetically active pigment systems (Sect. 3).

In concluding this general survey, one final remark should not be omitted. The presentation given here and later on has a slightly subjective character. This seems both inevitable and indicated, because a discussion covering every viewpoint and every type of observation would largely exceed the space available for this chapter. The author thinks his presentation to be in accordance with the observations and views of Van Niel, Calvin, and others. For a discussion of certain points in a somewhat different manner, the reader might consult Franck's survey articles (Franck, 1949, 1951<sup>2</sup>).

### 3. THE PHOTOSYNTHETIC APPARATUS AND PRELIMINARY REMARKS ON ENERGY TRANSFER

Chloroplasts were first recognized long ago. Leeuwenhoek, according to Weier (1938), may have been the first to observe and to describe them. More regular observations began about the end of the eighteenth century, simultaneously with the start of the study of photosynthesis. Figure 5-1, reproduced here from Schleiden's textbook of 1845, indicates that careful observations were already being made. Chloroplasts are also pictured and described in Jussieu's textbook of about the same time. The view was held by then that chlorophyll in the cell is deposited upon

<sup>2</sup> The author wishes to thank Professor Franck for his kindness in showing him this discussion in manuscript.

rather indefinite particles, leading to "chlorophyll grains" of various shapes. It was later recognized that they are specifically differentiated parts of the protoplasm, and their genesis, especially in developing cells of higher plants, was carefully studied by von Mohl and by Gris (see Sachs, 1874), Hofmeister (1867), and others. Sachs stressed that extraction of chlorophyll by lipophilic solvents demonstrates that chloroplasts

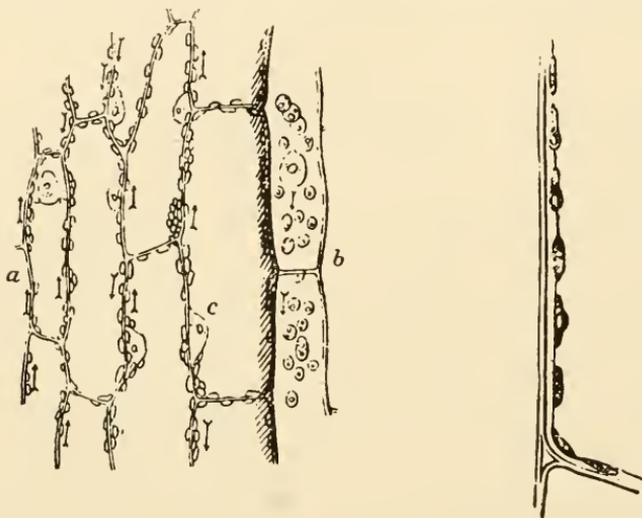


FIG. 5-1. An early picture of chloroplasts (*Chlorophyllkörnchen*: grains of chlorophyll) in the leaf of *Vallisneria spiralis*. (From Schleiden, 1845.)

consist of two parts: the pigment and its protoplasmic bearer, a distinction that had been made earlier by Treviranus in 1814 (cf. Zirkle, 1926). Sachs (1874, p. 46) states that chlorophyll is present in only minute amounts and that, after extraction, the protoplasmic part keeps its shape

and volume as "a solid, soft body which contains at best small vacuoles and in which the coloring matter sometimes is unequally distributed." Meyer in 1883 and Schimper in 1885, as is well known, first advocated a structure with dark "grana" in a lighter-colored "stroma," but for a long time the common opinion was that chlorophyll is homogeneously distributed in the chloroplast. According to Weier (1938), healthy and vital chloroplasts may be either granular or homogeneous. About 1935 the idea that chloroplasts often have a granular structure was revived, especially by the work of Heitz (1936) and of Doutréigne (1935). The latter presented photomicrographs of grana in chloroplasts in intact leaves of water plants in their natural medium (Fig. 5-2). Frey-Wyssling (1948) holds the view that in the chloroplasts, observed as homogeneous,

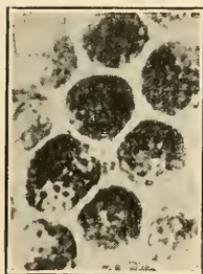


FIG. 5-2. Grana in chloroplasts of *Cabomba aquatica* in blue light. (From Doutréigne, 1935.)

the grana may be submicroscopic or may exhibit no optically visible phase boundaries and that they may become visible under certain conditions by "coarsening," which he does not consider as due to artifacts.

Electron-microscope studies have indicated that the grana are truly morphological units. According to Granick (1949), a mature spinach chloroplast contains 40-60 grana, about 6000 Å in diameter and 800 Å thick, embedded in the protein-containing matrix. He holds that the protein remaining after extraction of the grana with methanol makes up less than half the original material. Recently, Thomas *et al.* (1952) also made an electron-microscope study of spinach chloroplasts and grana. They confirmed the earlier findings of a membrane around the chloroplast, and by applying both pepsin and lipase digestion they demonstrated that this membrane, like the stroma, contains both proteins and lipids. The proteinaceous framework in which the grana are suspended shows a "thread-globule" structure. The globules contain substances that have a larger electron-scattering power than those found in the threads. After digestion of the proteins a spongy lipid mass remains. According to these authors the grana are also surrounded by a protein-lipid membrane. They confirm observations of Frey-Wyssling and Mühlethaler (1949) that grana consist of protein discs separated by lipids.

The renewed study of the grana has led to the view that they are the only seat of the chlorophyll. Evidence for this is contained in an observation by Jungers and Doutreligne (1943), who observed green grana and a white matrix against the background of starch grains in amyloplasts of potato exposed to light. Metzner (1937) succeeded in demonstrating chlorophyll fluorescence of grana while the stroma remained dark.

The available evidence suggests a structure of the granum of the type already proposed by Hubert in 1935 for the entire chloroplast. According to this suggestion, hydrophilic protein layers alternate with lipophilic layers, in which the pigments and lipids, e.g., cholesterol molecules, occur in a definite arrangement and have a definite orientation with respect to the protein. Frey-Wyssling (1948) estimates that a granum contains 20-30 parallel fatty layers, each having a thickness of about 50 Å, separated by aqueous protein layers having thicknesses of about 250 Å.

The view has developed that, in the chloroplasts, chlorophyll is bound to protein. An important argument for this was derived from spectrum studies which showed that in the living cell the red absorption maximum was at a longer wave length than in any solvent. This was much more so in the bacteriochlorophyll of the purple bacteria, where the difference may be as much as 100 m $\mu$  (Katz and Wassink, 1939). Extraction of bacteriochlorophyll from various strains yields solutions with an absorption maximum at approximately 770 m $\mu$  in ethanol, whereas the maxima in the living bacteria are found at various places between 800 and 900 m $\mu$

(Fig. 5-3a, b). A very large shift upon extraction is also found in the absorption maximum of the green pigment of green sulfur bacteria (Fig. 5-4). The absorption maxima in the red and near-infrared regions of aqueous colored extracts from ground cells of either green plants or the photosynthetic bacteria are at practically the same positions as in the

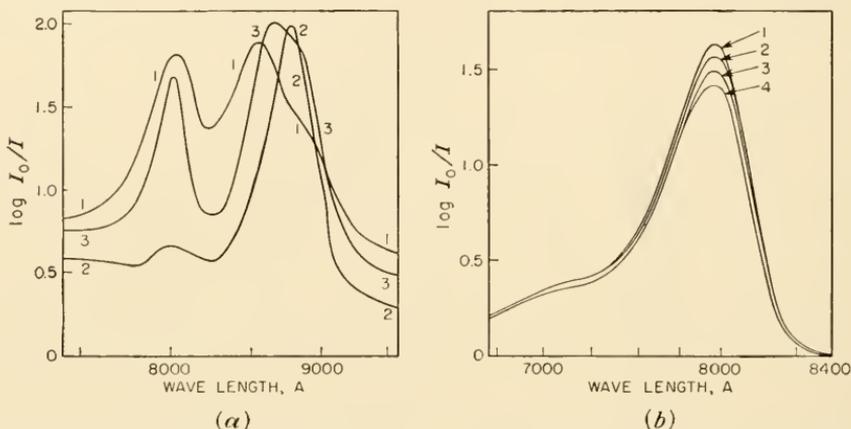


FIG. 5-3. (a) Infrared absorption spectra (of bacteriochlorophyll-protein complexes) of some purple bacteria, measured in the living cells. (1) *Chromatium*, strain D; (2) *Rhodospirillum rubrum*, strain 1; (3) *Rhodovibrio*, strain 1. (From Wassink, 1942; data from Wassink et al., 1939.)

(b) Infrared absorption spectra of extracted bacteriochlorophyll from some purple bacteria (in ethanol). (1) *Chromatium*, strain D; (2) *Rhodospirillum rubrum*, strain 1; (3) *Rhodovibrio*, strain 1; (4) *Phaeomonas varians*, strain 4. (From Wassink, 1942; data from Wassink et al., 1939.)

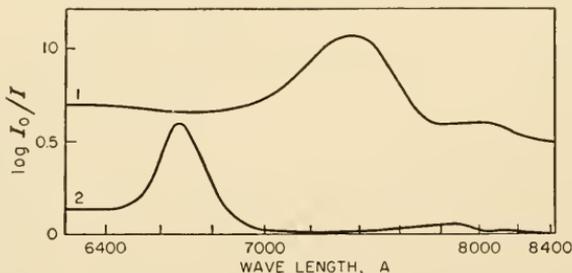


FIG. 5-4. Infrared absorption maximum of green bacteria (bacterioviridin) (upper curve), and its shift upon ethanol extraction (lower curve). (From Wassink, 1942, according to Katz and Wassink, 1939.)

living cells (Fig. 5-5a, b). The pigmented material does not precipitate with moderate centrifugal force. Electrophoretic movement is shown in an electric field, which movement at a certain pH is reversed (Katz and Wassink, 1939). In aging cultures of purple bacteria a colored material sometimes extrudes from the cells and remains suspended in the nutrient medium. Its absorption spectrum is quite similar to that of the living cells and to that of the extracts obtained by grinding.

The observations on purple bacteria suggest that in the living cells bacteriochlorophyll may occur as different pigment-protein compounds, each characterized by a different infrared absorption maximum (Wassink *et al.*, 1939). Association of the pigment with protein is now generally accepted as essential for photosynthesis. A function of the protein may be to conduct the energy absorbed by the pigment to energy acceptors acting chemically in photosynthesis. The pigment-protein complex from green cells was called "phylochlorin" (Mestre, 1930) or "chloroplastin" (Stoll, 1936). The present writer proposed that any colored pigment-protein complex from plant cells be called "chromophyllin" and that the photosynthetically active one from green plant cells be called "chlorophyllin" (Wassink, 1948b), a terminology related to that used by Engel-

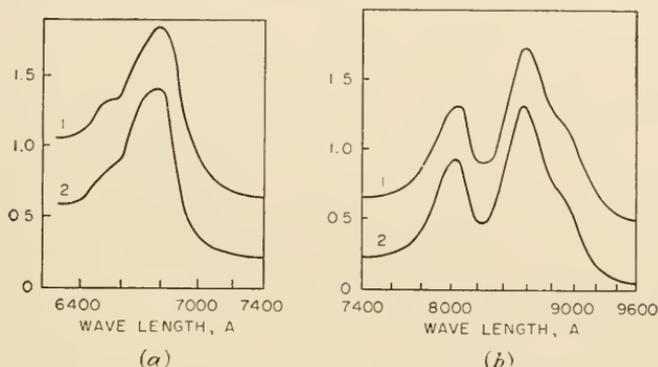


FIG. 5-5. (a) Red absorption maximum of chlorophyll in *Chlorella* cells (curve 1) and of the cell-free chlorophyllin (curve 2). (From Wassink, 1942, according to Katz and Wassink, 1939.)

(b) Infrared absorption maximum of bacteriochlorophyll in *Chromatium*, strain D, cells (curve 1) and cell-free bacteriochlorophyllin (curve 2). (From Wassink, 1942, according to Katz and Wassink, 1939.)

mann (1883). Stoll and coworkers (Stoll and Wiedemann, 1939; Stoll *et al.*, 1941) showed that the chlorophyllin in suspension contains carotenoids, probably the majority of the carotenoids present in the plastid in the living state. This also holds for the spontaneously extruding as well as for the artificially prepared chromophyllin from the purple bacteria; these chromophyllins contain bacteriochlorophyll and red carotenoids.

Blue-green algae, also, under certain conditions extrude a blue or purple compound in much the same way as described for the purple bacteria. This compound, phycocyanin, is a protein from which the splitting of the chromophoric grouping is very difficult (Lemberg, 1928, 1930, 1933). The breaking of the bond between pigment and protein is much easier in the chlorophyllin (and also in the bacteriochlorophyllin) than it is in the phycocyanin. The spontaneous extrusion of phycocyanin suggests that its protein differs from that of the chlorophyll-carotenoid complex, which under similar conditions remains inside the

cells. Recent observations on sensitized fluorescence suggest that, nevertheless, in the intact cell a close connection exists between chlorophyllin and phycoeyanin.

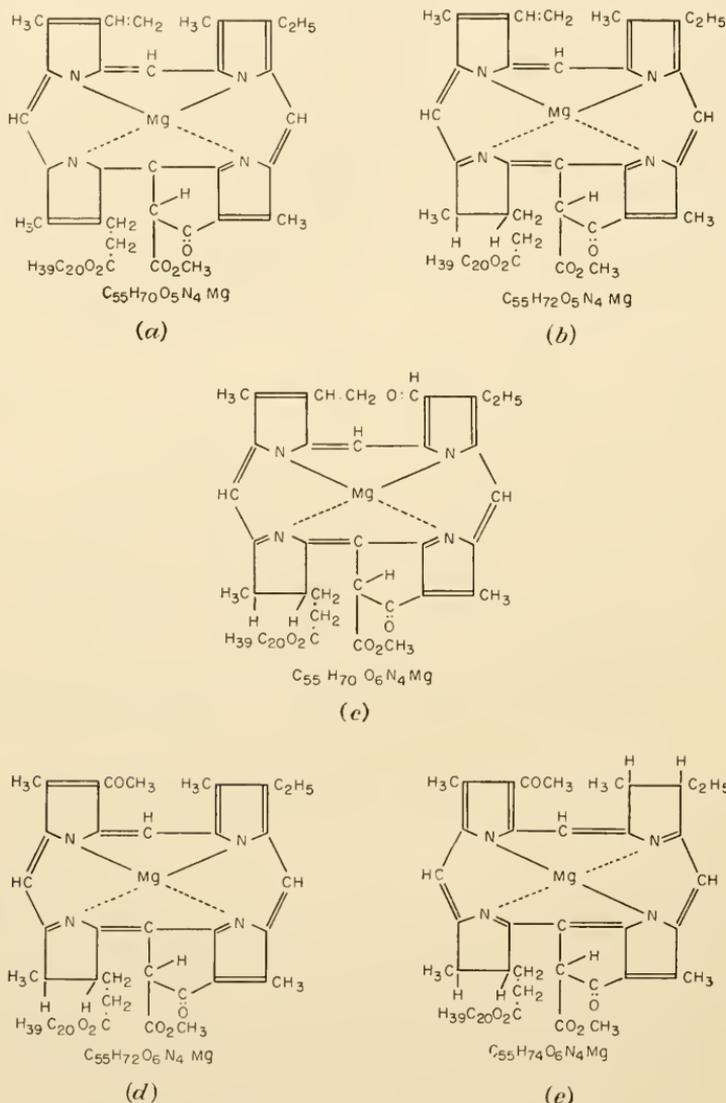


FIG. 5-6. Formulas of various chlorophylls. (a) Protochlorophyll; (b) chlorophyll a; (c) chlorophyll b; (d) bacterioviridin; (e) bacteriochlorophyll. (After Fischer and Orth, 1940.)

The energy absorbed by some carotenoids (as will be discussed later) is active in photosynthesis. As far as is known, these carotenoids act through chlorophyll as an intermediate since they also give rise to chlorophyll fluorescence (Dutton and Manning, 1941; Dutton *et al.*, 1943; Wassink and Kersten, 1946-1948). An important question is whether

the attachment to the chromophyllin for active carotenoids is different from that for inactive ones. In the case of the fucoxanthin of diatoms, evidence has been obtained indicative of a distinct binding of this active carotenoid to protein. Fucoxanthin shows a shift in absorption upon extraction, which is the main reason that the ethanol extract from the brown diatoms is green. Also, cells warmed to 70°C turn green, which

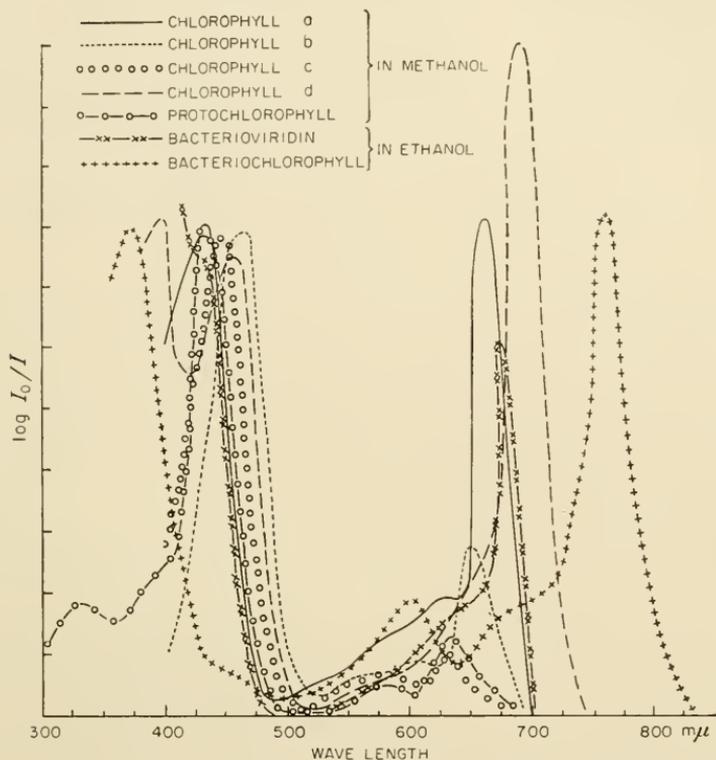


FIG. 5-7. Absorption spectra of various chlorophylls. (*Chlorophyll a, b, and c* from Strain and Manning, 1942; *chlorophyll d* from Manning and Strain, 1943; *protochlorophyll* from Koski and Smith, 1948; *bacterioviridin* from Katz and Wassink, 1939; *bacteriochlorophyll* from Manten, 1948).

very probably has to be interpreted as a change in the chromophyllin by denaturation of the protein (Wassink and Kersten, 1946-1948).

The formulas of some major types of chlorophyll (including protochlorophyll and bacterioviridin) and of bacteriochlorophyll (cf. Fischer and Orth, 1940) are shown in Fig. 5-6. Some absorption spectra from the literature are collected in Fig. 5-7. The chief absorption maxima of the various chlorophylls are situated in much the same regions, but the relative heights of the red maxima differ greatly. The maxima of bacteriochlorophylls are situated rather differently; the spectrum is much wider (cf. Manten, 1948). This appears to be due to the hydrogenation of a double bond between C<sub>3</sub> and C<sub>4</sub> in the pyrrol-methine nucleus of

the molecule, which, of course, strongly alters the system of conjugated double bonds. The rest of the molecule is nearly the same as that of chlorophyll a. A bacteriochlorophyll b is not known so far.

In green-plant cells the chloroplasts contain chlorophyll a and b and numerous yellow carotenoids. This photosynthetic system is found in green flagellates, green algae, and the Cormophyta. A few deviations from this distribution are known, one of which is the absence of chlorophyll b in *Vaucheria* (Seybold *et al.*, 1941). Chlorophyll a is much more widespread, being found in all nonbacterial photosynthetic organisms. It is associated with chlorophyll c (Strain and Manning, 1942; Wassink and Kersten, 1946-1948) in diatoms and brown algae, both of which contain the carotenoid fucoxanthin. The close relation between the photosynthetic pigment systems of these fairly divergent groups of algae is remarkable. A chlorophyll d was found in small amounts in red algae (Manning and Strain, 1943). Strain (1949) reported also on a chlorophyll e, found along with chlorophyll a in the yellow-green alga *Tribonema* spec. The blue-green and red algae contain a phycobilin pigment as well as the chlorophyll complex (including carotenoids). It is remarkable that the few groups of photosynthetic bacteria have developed two pigment systems that seem mutually independent and also widely divergent from the pigment system of all "chlorophyll plants." The green bacteria contain bacterioviridin, a compound which is considered to be 2-acetyl (nonvinyl) chlorophyll a (Fischer and Orth, 1940) and which, in extracts, has an absorption spectrum closely similar to that of chlorophyll a (Fig. 5-7). In the living cell its red absorption maximum is shifted about 70 m $\mu$  toward the infrared (Fig. 5-4). Sulfur and nonsulfur purple bacteria contain bacteriochlorophyll in various states of association with proteins. The photosynthetic bacteria also contain carotenoids, which, in the purple bacteria, are responsible for the color of the organisms.

The photosynthetic bacteria, as was mentioned earlier, cannot carry out the reaction summarized in Eq. (5-1). They preferentially substitute sulfur compounds for oxygen compounds and in part may use certain organic compounds or molecular hydrogen as reductors.

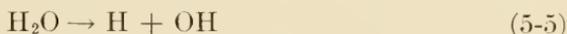
All photosynthetic organisms may show a fluorescence spectrally connected with the long-wave absorption band of the chlorophyllous pigment. As far as the writer knows, fluorescence of green bacteria has not yet been demonstrated, but no doubt it will be found. It is generally assumed that the average fluorescence wave length represents an excitation level of the pigment system from which the chemical-energy consumption also originates. This implies that smaller energy quanta are at the disposal of the cell in the photosynthetic systems of the bacteria than in those of green plant cells. The supposition that this difference has something to do with the inability of the bacteria to obtain the energy required for

Eq. (5-1) is tempting. It is, however, a lack in our present-day knowledge of photosynthesis that we are largely unfamiliar with the role that the chemistry of the pigment and its association with protein play in photosynthesis.

Certain reactions of purple sulfur bacteria, e.g.,



have hardly any over-all energy requirement. They nevertheless require a considerable amount of light energy (at least 1 quantum per hydrogen atom transferred) for their completion. This strongly suggests that in all types of photosynthesis there is a partial reaction that uses a compound stoichiometrically related to the input of light quanta. It has been supposed that this reaction is of the type



in both green-plant cells and colored algae and in bacteria. The present writer is not convinced of the correctness of this view, as will be discussed later (cf. also Wassink, 1947).

Before the discussion of the photosynthetic mechanism is continued, some further details will be given about the fate of the light energy after absorption in the pigment system of the photosynthetic cell. When a light quantum is absorbed in chlorophyll, it gives rise to an excited state of the chlorophyll molecule. Chlorophyll shows two chief absorption bands in the visible part of the spectrum, namely, in the blue and in the red, with a region of lower absorption coefficients in the green and yellow. Quanta of various energy contents ultimately yield the same excited state of chlorophyll, namely, the state corresponding to the red absorption maximum (the one of lowest energy shift). This is obvious from the fact that chlorophyll fluorescence shows a relation only to the red absorption band and that the fluorescence spectrum is independent of the wave length of the incident light. Chlorophyll may lose its excitation energy in various ways: (1) by transfer to a compound that is able to use the energy in a (photo)chemical process, (2) as heat, or (3) as fluorescence. Fluorescence will be the path if an excited state has escaped annihilation by paths 1 and 2. Under physiological conditions, chlorophyll fluorescence in plant cells is weak, of the order of a few tenths of 1 per cent of the incident light. Since no appreciable fraction of the available energy enters into the fluorescence phenomenon, fluorescence can be used as a sensitive indicator for the available concentration of excited chlorophyll and for the changes this concentration undergoes with changes in path 1 or 2. This reasoning has been the basis of numerous studies regarding chlorophyll fluorescence in photosynthesizing cells and the relation of fluorescence to the process of photosynthesis. The general conclusion from this work is that close connections between photosynthe-

sis and chlorophyll fluorescence show up when the process limiting the rate of photosynthesis is closely connected with the energy transfer from the chlorophyll. This obtains when the rate-limiting process influences the available concentration of energy acceptor. The erroneous assumption has often been made that a limitation of the rate of photosynthesis necessarily inhibits the energy transfer. Limitation of photosynthesis has been found, however, to be accompanied by either unchanged, decreased, or even enhanced transfer of energy. In these cases unchanged, increased, or decreased fluorescence yields, respectively, are observed.

The carotenoid fucoxanthin in diatoms was the first pigment for which energy transfer to chlorophyll a without loss was proved. It has been found that the energy absorbed by fucoxanthin leads to fluorescence of chlorophyll with the same yield as the energy absorbed directly by chlorophyll (Dutton and Manning, 1941; Dutton *et al.*, 1943; Wassink and Kersten, 1946-1948). This energy is also equally effective in photosynthesis.

Extensive work of Duysens (1951) indicates that a similar situation exists in many other cases. By a quantitative study of fluorescence yield in relation to the wave length of the incident light ("action spectra for fluorescence") and of fluorescence spectra, he showed that energy is transferred from chlorophyll b to chlorophyll a and from certain carotenoids of purple bacteria to the bacteriochlorophyll-protein complexes. It is of special interest that in *Chromatium*, strain D (the strain used generally by the Utrecht Biophysical Group; cf. Wassink *et al.*, 1942), energy transfer could be demonstrated from the bacteriochlorophyll-protein complexes with higher excitation levels to the complex with the lowest excitation level (the longest wave-length absorption). According to Duysens, the efficiency of the carotenoids for exciting fluorescence of the bacteriochlorophyll-protein complex (with absorption at about 890 m $\mu$ ) is of the order of 40 per cent. In *Chlorella* he found transfer from chlorophyll b to chlorophyll a with an efficiency of almost 100 per cent.

A very peculiar situation has been recorded in red algae. Blinks *et al.* (1949) and Haxo and Blinks (1950), in detailed and extensive studies, showed that in these algae chlorophyll and the carotenoids were much less efficient for oxygen evolution than the phycobilins. For the light absorbed chiefly by the bilins, an efficiency of about  $\frac{1}{12}$  (one molecule of oxygen evolved for 12 quanta absorbed) was found as compared with an efficiency of only  $\frac{1}{40}$  to  $\frac{1}{50}$  for the light absorbed by chlorophyll or the carotenoids. Most of the energy absorbed by the chlorophyll-carotenoid complex in these algae is apparently wasted (Fig. 5-8). Duysens (1951) also presents some very interesting observations on this energy transfer. The fluorescence of chlorophyll a per quantum absorbed by phycoerythrin (in *Porphyra*) is stronger than its fluorescence per quantum absorbed by itself! The same was found in other species. An unknown pigment with a fluorescence maximum at about 725 m $\mu$ , however, showed the reverse

behavior, fluorescing much more strongly with light absorbed by chlorophyll a than with that absorbed by the bilins. This suggests that chlorophyll a in the red algae has a low photosynthetic activity because part of it is connected with the 725-m $\mu$ -fluorescent pigment, which easily takes over its energy. Duysens obtained some indications that this unknown pigment might be chlorophyll d. Since the absorption of chlorophyll d in the organisms used is not very evident in comparison with its fluorescence, the fluorescence must have an unusually high yield.

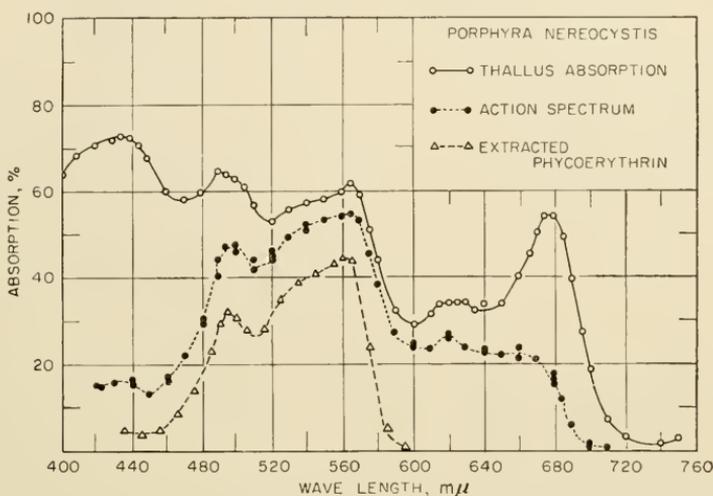


Fig. 5-8. Absorption spectra of pigments, and spectral sensitivity of photosynthesis in a red alga. (From Haxo and Blinks, 1950.)

Duysens concludes, from his quantitative estimates of the yield of chlorophyll a fluorescence, that in red algae phycoerythrin and phycocyanin transfer their excitation energy to chlorophyll a with an efficiency probably above 80 per cent. This emphasizes the dual character of the chlorophyll a in these plants. Part of it is active in photosynthesis and accepts the bulk of energy absorbed by the other pigments; the other part is inactive in photosynthesis, being coupled with the unknown, strongly fluorescent pigment and inaccessible to the energy absorbed by other pigments.

Duysens holds the view that excitation energy flows to the chemical stage of the photosynthetic process only via chlorophyll a and, in bacteria, via the bacteriochlorophyll complex with the lowest excitation level.

Green bacteria have not been studied.

The importance of fluorescence spectra and action spectra for fluorescence in the study of the mechanism of energy transfer, so clearly demonstrated in these investigations, was theoretically discussed earlier by Wassink (1948b) (cf. also Sect. 5).

Haxo and Blinks (1950) found that the greater part of the carotenoids

are active in photosynthesis in the green alga *Ulva*. Red algae, which, owing to environmental conditions, had developed little phycobilin, showed higher photosynthetic efficiency of chlorophyll a.

Some further discussion on energy transfer will be found in Sect. 5.

#### 4. EVIDENCE FOR THE MECHANISM OF PHOTOSYNTHESIS AS OUTLINED IN SECT. 2

##### 4-1. OBSERVATIONS AND CONSIDERATIONS OF A COMPARATIVE BIOCHEMICAL NATURE

Van Niel (1940) points out that certain purple bacteria can act on the same hydrogen donors in two different ways, namely, in the dark with oxygen as the ultimate hydrogen acceptor and in the light with carbon dioxide as the acceptor. He also points out that both in light and in darkness various hydrogen donors may be consumed simultaneously and apparently independently, so that the total rate of conversion is the sum of the rates obtained for each hydrogen donor. These observations make it seem unlikely that radiant energy brings about a special activation of the hydrogen donors in photosynthesis.

It is well known, moreover, that many organisms can reduce carbon dioxide in the dark, so that a special photoactivation is doubtful for carbon dioxide. Van Niel states: ". . . The probability has to be seriously considered that the reduction of this substance takes place only after its incorporation into some organic molecule, and as a result of reducing systems active in the dark but generated in the light." This conclusion is in accord with conclusions previously reached by Ornstein *et al.* (1938) and by Wassink and Katz (1939) from comparative studies of chlorophyll fluorescence and photosynthesis, and with more recent results by Calvin and Benson (1948).

These facts give support to the postulation of the partial processes (1) and (3), as formulated in Sect. 2, and especially to their separation from the photochemical reaction (2).

##### 4-2. THE COMBINED STUDY OF PHOTOSYNTHESIS AND CHLOROPHYLL FLUORESCENCE

Light absorption in the pigment-protein complex, as was mentioned in Sect. 3, leads to a certain concentration of excited chlorophyll molecules, made evident by fluorescence, which is controlled by the input of light quanta and the transfer of excitation energy. Other molecules may use this transferred energy in chemical reactions or convert it into heat. Since the chief conversions of light energy into chemical energy occur in the process of photosynthesis, it is plausible to assume an intimate connection between changes in the rate of photosynthesis and changes in the amount of chlorophyll fluorescence. The suggestion has been made

that they should be mirror images (Müller, 1874; Kautsky and Hirsch, 1934), but this turned out to be too simple. It can easily be seen that those changes in photosynthesis which influence directly or indirectly the energy transfer from chlorophyll will have an influence on the intensity of fluorescence. It is, however, not strictly necessary for all changes in the rate of photosynthesis to be accompanied by changes in fluorescence, since every type of inhibition of the photosynthetic process does not necessarily influence the energy transfer from chlorophyll. Numerous studies have been made in recent years from the point of view of the connection of chlorophyll fluorescence and photosynthesis. They were initiated by the extensive investigations of Kautsky and Hirsch (1934), who studied especially fluorescence induction phenomena. Comparative studies of fluorescence and photosynthesis were undertaken by McAlister and Myers (1940), by Franck *et al.* (see, for example, Franck and Gaffron, 1941; Franck, 1949, 1951; Shiao and Franck, 1947), by Ornstein *et al.* (1938), and by Wassink *et al.* (see, for example, Wassink *et al.*, 1938; Wassink and Katz, 1939; Wassink *et al.*, 1942; Wassink and Kersten, 1943-1945). Wassink (1951b) gave a detailed discussion of the work from these various groups.

The facts which bear on the mechanism of energy transfer and which permit distinction between partial processes of photosynthesis closely related to the energy transfer and those which are unrelated will now be considered. Some results obtained with purple sulfur bacteria (*Chromatium*, strain D) are of special interest. Under normal conditions of photosynthesis, with excess hydrogen donor and excess carbon dioxide, the fluorescence is increased at light intensities for which photosynthesis shows light saturation (Wassink *et al.*, 1942). Under conditions of limited supply of hydrogen donor, the change in amount of fluorescence occurs at lower light intensities (Fig. 5-9).

It has been concluded from these observations that the presence of a hydrogen donor is essential for the transfer of energy from bacteriochlorophyll excited by light. When excess hydrogen donor is present, lowering of temperature affects fluorescence in much the same way as

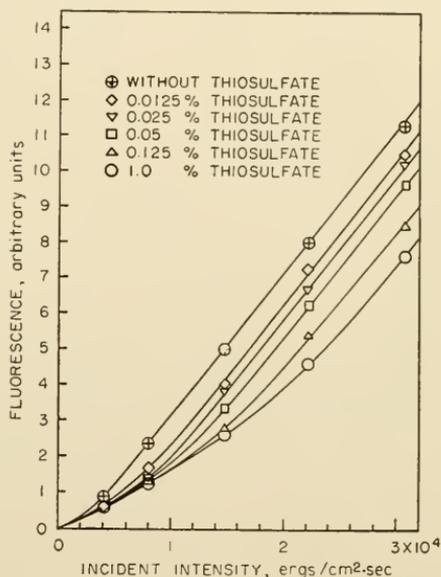


Fig. 5-9. Fluorescence of *Chromatium*, strain D, as a function of hydrogen-donor concentration ( $N_2 + 5$  per cent  $CO_2$ , phosphate buffer pH 6.3, 29°C). (From Wassink *et al.*, 1942.)

does lowering of the concentration of the hydrogen donor. This led to the conclusion that the hydrogen donor is not active in the process of energy transfer as such but influences this transfer only via a temperature-sensitive dark reaction.

If photosynthesis is observed by measuring gas exchange at various incident intensities of light, limitation of the concentration of the hydrogen donor yields curves showing a typical Blackman inhibition; i.e., photosynthesis is strongly inhibited at high intensities and only slightly or not at all at low light intensities. A similar situation is found if carbon dioxide is available in only limited amounts (Fig. 5-10*a, b*). This means

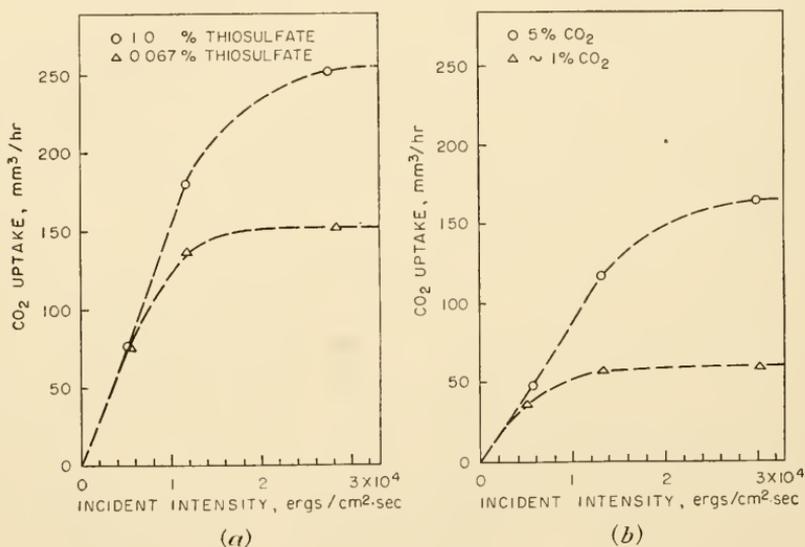


FIG. 5-10. (a) The influence of the concentration of the hydrogen donor (sodium thiosulfate) upon the rate of photosynthesis in *Chromatium*, strain D ( $N_2 + 5$  per cent  $CO_2$ , phosphate buffer pH 6.3,  $29^\circ C$ ). (From Wassink *et al.*, 1942.)

(b) The influence of the concentration of  $CO_2$  upon the rate of photosynthesis in *Chromatium*, strain D ( $N_2 + CO_2$ , sodium thiosulfate 1 per cent, phosphate buffer pH 6.3,  $29^\circ C$ ). (From Wassink *et al.*, 1942.)

that both the hydrogen donor and carbon dioxide react in dark chemical reactions and not specifically in the photoactive part of the process. In this connection it is of special interest that the way in which fluorescence of bacteriochlorophyll reacts upon the attainment of light saturation in photosynthesis depends on the cause of the light saturation. Limited availability of the hydrogen donor causes a strongly increased fluorescence yield at high light intensities. On the other hand, limited availability of carbon dioxide leaves the fluorescence yield practically unaltered (Fig. 5-11). There is only a slight increase in yield at medium light intensities, and at high intensities the yield often is even somewhat decreased.

These observations reveal the important fact that the dark reactions

in which the hydrogen donors and carbon dioxide react are different and, especially, that they have different relations to the energy-transfer system. Limited availability of the hydrogen donor directly affects fluorescence, i.e., energy transfer, whereas limited availability of carbon dioxide affects energy transfer much more indirectly, if at all. The observations quoted thus proved directly the separation of reactions (1) and (3), as given in Sect. 2. Indirectly they furnish evidence for the separation from process (2), the light reaction as such, since limitations in either (1) or (3) have the character of Blackman reactions.

The following explanation has been suggested (Wassink *et al.*, 1942) for the slight influence of withdrawal of carbon dioxide upon fluorescence. It is quoted here in some detail since it illustrates the interaction of the partial processes postulated in Sect. 2 (p. 299).

At low light intensities the capacity of the system that transforms the hydrogen donors [system (1), Sect. 2] is sufficient to prevent empty places in the system of energy transfer (system 2). The number of places in the latter system that are occupied with activated energy acceptor, under stationary conditions of illumination with light of low intensities, appears to be small even in the absence of carbon dioxide. This means that the time of annihilation of an activated state is small, also in the absence of carbon dioxide, as compared with the intervals

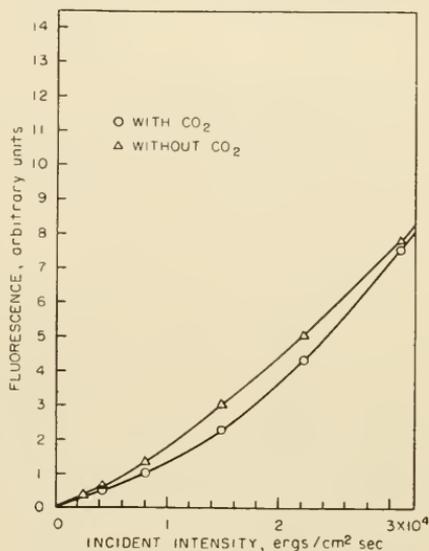


FIG. 5-11. Fluorescence of *Chromatium*, strain D, as influenced by the presence or absence of CO<sub>2</sub> (N<sub>2</sub> + 15 per cent H<sub>2</sub> ± 5 per cent CO<sub>2</sub>, phosphate buffer pH 6.3, 29°C). (From Wassink *et al.*, 1942.)

at which light quanta lead to activation at a certain spot. The energy-transfer process, under these conditions, is limited only by the number of absorbed quanta.

At medium light intensities this situation gradually changes. Obviously, in the absence of carbon dioxide, the system of energy transfer is not capable of getting rid of all activated molecules before the next quantum hits the same area. This leads to an increased concentration of excited bacteriochlorophyll, which cannot transfer its excitation energy to an acceptor molecule rapidly enough because an activated molecule still occupies the place. The result is an increased fluorescence, which is actually found at medium incident light intensities. The process of energy transfer now is limited by the number of places ready to accept energy. Since the absence of carbon dioxide emphasizes the situation outlined, an indirect coupling may be accepted between the transfer of energy and the consumption of activated energy acceptor in process (3) of Sect. 2.

At high incident light intensities the capacity of system (1) of Sect. 2 will become a limiting factor for the energy transfer, especially if a sufficient amount of carbon dioxide is available. The annihilation of activated acceptor molecules appears unhampered, and with increasing light intensity the energy transfer gradually becomes limited by the supply of molecules ready to accept energy. This leads to an increase in the concentration of excited bacteriochlorophyll, which gives rise to an increased fluorescence yield. It seems, furthermore, that activated energy acceptor, which does not react in system (3) of Sect. 2, is reconverted after some time into nonactivated acceptor, ready to accept energy again. This can be considered the reversal of a reaction of the type



which may occur if FH cannot be reconverted to F in the usual way owing to lack of carbon dioxide. It thus seems that the amount of energy acceptor at the system of energy transfer is less easily lowered at high light intensities in the absence of carbon dioxide than in its presence. This leads to a lower stationary concentration of excited bacteriochlorophyll and thus to a lower fluorescence (cf. also Wassink *et al.*, 1942, p. 323).

Another set of observations on fluorescence which are important in understanding the mechanism of photosynthesis will now be considered. Many observers, e.g., Kautsky and coworkers and Wassink and coworkers, have found that upon illumination of a photosynthetic system, such as a leaf or a suspension of algae, fluorescence undergoes a number of distinct and reproducible changes. Some observations made by Wassink and Katz (1939) on the fluorescence of algal suspensions of known density under controlled conditions, which recently received renewed attention because of their bearing on the mechanism of photosynthesis, are of interest (Calvin and Benson, 1948). Wassink and Katz (1939) found that a suspension of *Chlorella* when illuminated under nitrogen at first shows a much higher fluorescence than when illuminated under air. After some 20 min the fluorescence of the suspension in nitrogen reaches practically the level of that in air. These observations indicate that in *Chlorella* a reduced state is connected with a higher fluorescence than a more oxidized one. It is in accordance with this explanation that the decay of fluorescence observed under prolonged illumination—also in air but much stronger in nitrogen—is prevented by cyanide (Fig. 5-12). Obviously this is due to inhibition of oxygen production by cyanide. In general, the fluorescence-time curve consists of a rise, followed by a decay. The rise is insensitive to cyanide. A suspension of *Chlorella* in the presence of a concentration of cyanide which is just sufficient to stop photosynthesis shows upon illumination a rise of fluorescence above its starting point, after which the fluorescence intensity remains unchanged for at least 3 min. The rise is steeper with higher light intensities or with lower oxygen tensions. These facts have led to the conclusion that one of the most direct effects of light upon the photo-

synthetic apparatus is a reducing action in the immediate neighborhood of the chlorophyll (see Wassink and Katz, 1939, p. 152).

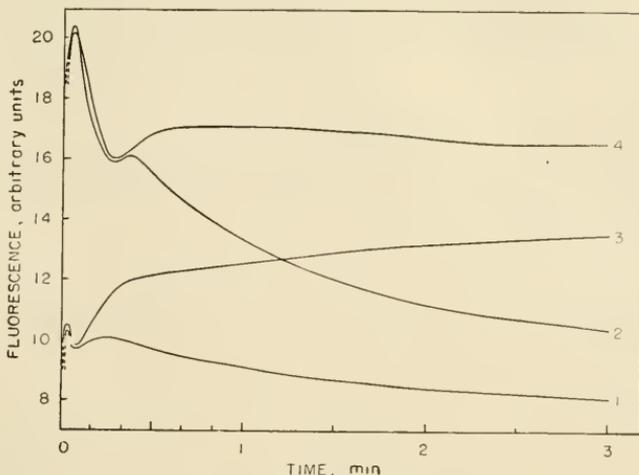


FIG. 5-12. Fluorescence-time curves of *Chlorella* suspensions. Gas phase: air (curves 1 and 3) or nitrogen (curves 2 and 4); normal (curves 1 and 2) or under complete inhibition of photosynthesis by cyanide (curves 3 and 4) (light intensity  $1.38 \times 10^4$  ergs/cm<sup>2</sup> · sec; Warburg Buffer No. 9, 29°C). (From Wassink and Katz, 1939.)

#### 4-3. TRACER EXPERIMENTS

By means of tracer experiments, Calvin and Benson (1948) independently reached the same conclusion as that given in the preceding section. They followed the absorption of  $C^{14}O_2$  by a suspension of *Scenedesmus* in darkness. These suspensions slowly take up  $C^{14}O_2$ , but if an illumination under a carbon dioxide-free atmosphere has preceded, a strong and rapid extra consumption of  $C^{14}O_2$  is observed in subsequent darkness. From this they concluded that green algae have the ability "to accumulate a certain amount of reducing power during illumination in the absence of carbon dioxide, which could later be used for the reduction of carbon dioxide" (*ibid.*, p. 476; see Fig. 5-13).

The previously mentioned experiments on fluorescence, as well as the tracer experiments just described, elucidate the nature of process (2) of Sect. 2, the light reaction proper. It appears to have the character of a

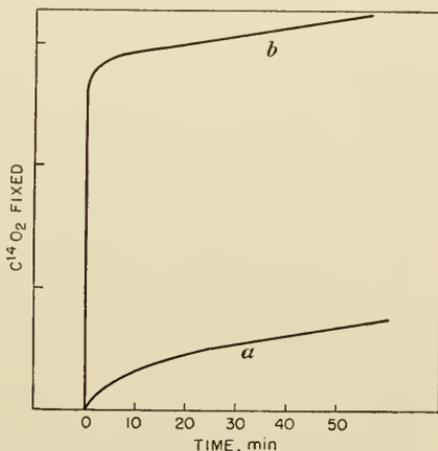
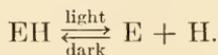


FIG. 5-13. Fixation of  $C^{14}O_2$  in *Scenedesmus* after dark period (a) and after pre-illumination in the absence of  $CO_2$  (b). (From Calvin and Benson, 1948.)

process producing a reducing agent, and it is tempting to assume that, in intervals between the impact of successive quanta on the same spots, dark reactions tend to reverse the situation, so that the system acts as a reversible redox system. The reaction cycle may be represented as follows:



The H, which may well be attached to some large group, may be considered active in the process of carbon dioxide reduction. The statement by Van Niel quoted in Sect. 4-1 is again pertinent.

An early isotope experiment that is still of interest is that of Pratt and Trelease (1938). They investigated the photosynthesis of *Chlorella* in deuterium oxide and found that the light-saturation value was much lower than in ordinary water, whereas no difference was observed in the light-limiting range. They concluded that water enters into a dark reaction. This is in agreement with results of the study of photosynthesis and bacteriochlorophyll fluorescence in *Chromatium*, which led to the conclusion that the hydrogen donor is connected with the transfer of energy by a dark process. The experiment of Pratt and Trelease might suggest a similar situation with regard to water and energy transfer in *Chlorella*. It should be remarked, however, that, as long as correlative fluorescence measurements are lacking, no definite conclusion can be drawn because of the great variety of effects that replacement of water by deuterium oxide may have on cellular metabolism.

At this point a brief discussion of the work on the path of carbon in photosynthesis carried out with tracer carbon, especially since 1946, is pertinent. Only those points which appear of direct interest for the general discussion of the mechanism of photosynthesis will be considered. Study of photosynthesis with the aid of carbon isotopes was started in 1938 by Ruben and his associates (Kamen, 1949). They were able at first to use only the short-lived isotope C<sup>11</sup>. From this work Ruben *et al.* concluded that a primary carboxylation reaction led to the formation of RCOOH, followed by a photochemical reduction in which carbon dioxide was assimilated with regeneration of the carbon dioxide acceptor RH. The formation of RCOOH from carbon dioxide and RH was assumed to occur in the dark. The presumed compound RCOOH was actually isolated later by Benson and Calvin (1947), when C<sup>14</sup> had become available, and was found to be chiefly succinic acid.

Studies with C<sup>14</sup> were carried on independently by Gaffron's group (e.g., Fager *et al.*, 1950) and by the group associated with Calvin and Benson (e.g., Calvin *et al.*, 1950). The former group, who were in close contact with proponents for the Franck-Herzfeld theory of photosynthesis, claimed to have evidence for a product of the primary action of the light on a carbon dioxide-containing compound which was unavailable

for the respiratory mechanism in the dark. This compound was found to be different from any known respiratory intermediate and could be transformed further only in light reactions (Brown *et al.*, 1948). It would thus have many of the properties of a "stabilized photoproduct," as required by the Franck-Herzfeld theory.

Quite different results were obtained by the group associated with Benson and Calvin. In an early stage of their investigation they made the previously discussed observations on the increased carbon dioxide uptake by carbon dioxide-free preilluminated suspensions of *Chlorella*.

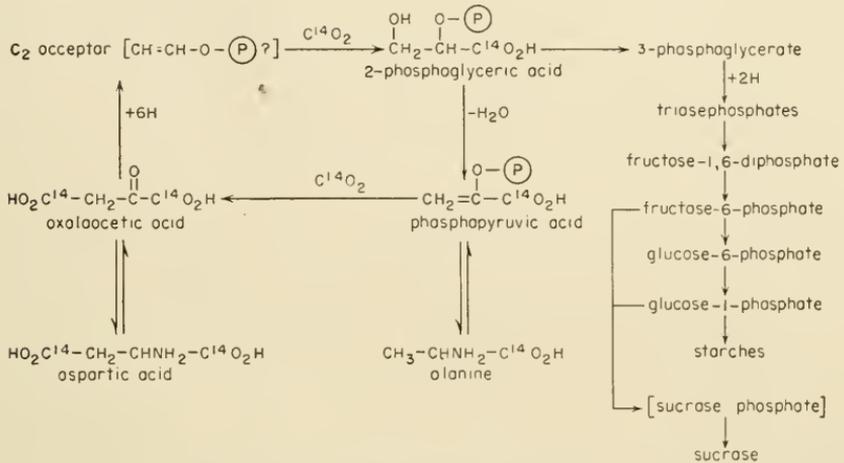


FIG. 5-14. The assumed path of CO<sub>2</sub> reduction in photosynthesis. (From Benson and Calvin, 1950.)

This led them to the assumption that reducing power is generated by the light and brought them surprisingly near to the conclusions and views derived earlier from fluorescence work by Wassink and Katz (1939). Moreover their assumption was consistent with Van Niel's deductions from comparative biochemistry. An important development of the work of Benson and Calvin was the discovery that, if cells in stationary conditions of photosynthesis (with C<sup>12</sup>O<sub>2</sub>) were allowed to take up C<sup>14</sup>O<sub>2</sub> for a few seconds, radioactivity is predominant in a few compounds, especially in 2- and 3-phosphoglyceric acid (Calvin, 1949). Phosphoglyceric acid was identified as the earliest labeled intermediate also in more recent work of the Chicago group (Fager *et al.*, 1950). The distribution of radioactivity in increased exposures to C<sup>14</sup>O<sub>2</sub> in the light led to the suggestion that the "path of carbon in photosynthesis" is chiefly a reversal of respiration. Benson and Calvin (1950) drew up a scheme (Fig. 5-14), the essence of which is a dicarboxylic cycle in which a two-carbon "acceptor" molecule is converted to a four-carbon compound by two successive carboxylations. Fat, protein, and carbohydrate syntheses use intermediates of photosynthesis from this cycle. The two-carbon

carbon dioxide acceptor that may have properties of the "reducing agent" is tentatively formulated as vinyl phosphate, and some results suggest that glycolic acid may participate in the formation of the carbon dioxide acceptor. More recent results suggest that the  $C_2$  compound is not generated from a  $C_1$ — $C_1$  condensation but is derived from  $C_3$  and  $C_4$  compounds (Calvin *et al.*, 1950, p. 531, and Bassham *et al.*, 1954\*).

The generation of a reducing power under carbon dioxide-free illumination has been questioned by Brown *et al.* (1948). They interpret the increased uptake, as found by Calvin *et al.* after preillumination in the absence of carbon dioxide, as being due to a depletion of carbon dioxide "stores" during the illumination. Benson and Calvin (1950) consider this improbable for two reasons: (1) uptake of carbon dioxide after carbon dioxide-free preillumination yields chiefly the same compounds as photosynthesis; (2) cells of *Scenedesmus*, preilluminated without carbon dioxide, take up carbon dioxide at a rate one hundred times greater than non-preilluminated ones. According to the view of Brown *et al.*, this would mean that the pressure of carbon dioxide inside the cells had decreased about one hundred times, i.e., to about 0.001 mm Hg during preillumination. This was considered improbable, and the conclusion was drawn "that the chief action of light was to produce reducing agent(s) and carbon dioxide acceptor(s)" (Benson and Calvin, 1950, p. 37). It should be pointed out, however, that it is difficult to estimate the lower level to which the pressure of carbon dioxide inside a carbon dioxide-free illuminated cell may fall. Wassink *et al.* (1951) observed that illumination of a *Chlorella* suspension in the absence of carbon dioxide may easily lead to precipitation of inorganic phosphate. Therefore, the result of Calvin and Benson's experiment may not be fully conclusive as to the generation of reducing power. Nevertheless, it is supported by various other kinds of evidence [collected, for example, by Wassink (1951a)], and it seems to offer the most rational explanation. For a further development of this discussion see Gaffron and Fager (1951).

Light intensity appears to influence the distribution between 2- and 3-phosphoglyceric acid as first products. *Scenedesmus* gives the same products in hydrogen-adapted photoreduction, and in photosynthesis. According to Calvin *et al.* (1950, p. 527), "the major difference between photosynthesis and photoreduction, then, lies in the mechanisms of hydrogen transfer and not in the path of carbon reduction by the reducing agents produced." As to the relation between photosynthesis and the depletion of reservoirs by preillumination, it is interesting that they found the proportion of radiocarbon in malic acid, aspartic acid, and alanine greater in the latter case than in short periods of photosynthesis. "Depletion of the malic acid ( $C_4$ ) and alanine ( $C_3$ ) reservoirs by preillumination (reduction to hexose) resulted in their restoration with labeled compounds as soon as a source of carbon dioxide became available." It should be observed that this conclusion is consistent with the view of Brown *et al.* (1948) discussed previously. If pre-

\* See Addendum.

illumination (under carbon dioxide-free conditions) is omitted, the dark uptake of carbon dioxide was only about 0.1–0.01 of that after preillumination. The products were also different; i.e., 95 per cent of the total carbon fixed was in malic and succinic acid, alanine, and some other acids. The labeling seems to indicate the reversibility of normal carboxylation reactions. According to Calvin *et al.* (1950, p. 528): “Those compounds labeled in the light and in preillumination experiments only are considered products of photosynthesis, while alanine, malic acid, and aspartic acid, labeled slowly in non-preilluminated dark experiments and much more rapidly in light and preilluminated dark experiments, are considered to be products of both photosynthesis and reversible respiration reactions.”

If the length of exposure is shortened, most  $C^{14}O_2$  is in the COOH of phosphoglyceric acid, which suggests that this substance is formed by carboxylation of a  $C_2$  compound (*ibid.*, p. 529). The activity of malic and aspartic acids in short exposures is also in the carboxyl groups. Together with the early appearance of labeled malic acid and phosphopyruvic acid, this suggests the conversion:



Thus carbon dioxide reduction in the light would involve two carboxylations ( $C_2 \rightarrow C_3$ ,  $C_3 \rightarrow C_4$ ). It is a very interesting finding that, although at high light intensities (400–10,000 ft-c) in short exposures chiefly phosphoglyceric acid is formed, below 50 ft-c chiefly malic acid arises. The variation is ascribed to a variation in the concentrations of the respective carbon dioxide acceptors with light intensity (*ibid.*, p. 530). The way the authors look upon the nature of the  $C_2$  compound is of interest. They considered it to be a highly reduced compound. “It would be formed readily in the presence of photochemically produced reducing power, but in the dark it would probably be formed only by reversal of the  $C_2 \rightarrow C_3$  carboxylation, and in the latter case subsequent rapid oxidative reactions might keep its concentration at a very low level.” The concentration of  $C_3$  carbon dioxide acceptor (which is probably phosphopyruvic acid) is maintained in darkness by glycolysis, whereas the concentration of  $C_2$  carbon dioxide acceptor is then low. This would explain why  $C^{14}O_2$  uptake in dark results in labeled  $C_4$  preferably and  $C^{14}O_2$  in light or after carbon dioxide-free preillumination results in labeled  $C_3$  (phosphoglyceric acid). The same holds for a comparison of low and high light intensities; with the high intensities the  $C_2$  carbon dioxide acceptor predominates, and phosphoglyceric acid arises as the chief labeled compound in short exposures.

These considerations show that Calvin *et al.* (1950) do not consider the  $C_2$  and  $C_3$  carbon dioxide acceptors to be identical with the reducing power generated during continuous illumination or carbon dioxide-free preillumination; they are “formed in the presence of photochemically produced reducing power” (p. 530). It is unlikely, for several reasons, that the  $C_2$  compound is generated by  $C_1$ — $C_1$  condensation. Traces of formaldehyde, formic acid, and acetic acid were found which, according to these workers, might well be artifacts. A good argument against direct synthesis of a  $C_2$  compound is that in brief experiments no significant amounts of labeled  $C_2$  compounds, as, for example, glycine and glycolic acid, are found and that in phosphoglyceric acid only a few per cent of the labeling is outside the carboxyl group. On the other hand, if plants, by brief illumination in the presence of  $C^{14}O_2$ , have produced labeled  $C_3$  and  $C_4$  compounds (covering

practically the total of radioactivity) and then are illuminated in the absence of carbon dioxide, labeling in  $C_3$  and  $C_4$  compounds decreases in favor of the formation of labeled glycolic acid and glycine. This not only raises another argument against a  $C_1-C_1$  condensation as a source of  $C_2$  compounds but favors the assumption of a  $C_2-C_3-C_4-C_2$  cycle, as mentioned earlier, in which  $C_4 \rightarrow C_2$ , since generation of  $C_2$  from  $C_3$  would be likely to yield products such as formic acid or formaldehyde and these were not observed. Calvin *et al.* carried out experiments to see whether malic acid is an intermediate in this cycle. They used malonate-treated *Scenedesmus*, which, after suspension in malonate-free buffer, was actively photosynthesizing, and exposed it to  $C^{14}O_2$  over a short period. Whereas radiocarbon in malic acid was greatly decreased as compared with normal cells, the other short-exposure products were practically unchanged. The labeling of  $\alpha$  and  $\beta$  carbon atoms of glyceric acid was the same. They concluded that, if phosphoglyceric acid is an intermediate in photosynthetic carbohydrate synthesis by reversing glycolysis, malic acid is not an intermediate but rather a carbon reservoir that is readily derived from some intermediate in photosynthesis. Neither fumaric nor succinic acid showed any special relation to photosynthesis. Oxaloacetic acid is thought to be a possible intermediate for the formation of the  $C_2$  carbon dioxide acceptor.

Late reports (Benson, 1951; Benson *et al.*, 1951) indicate the finding of sedoheptulosephosphate and ribulosediphosphate in a very early stage of  $C^{14}O_2$  fixation in photosynthesizing plants. Tentatively these products are suggested as participating in the system for regeneration of the  $C_2$  carbon dioxide acceptor. A critical survey of the photosynthesis research with  $C^{14}O_2$  was given by Utter and Wood (1951).

It is interesting that, if the external atmosphere contains 20 per cent oxygen, about ten times more labeled glycolic acid is formed than if there is only 1 per cent oxygen. Calvin *et al.* see two possible explanations: (1) the oxaloacetic acid is oxidized before splitting, or (2) a glycolic acid is formed by oxidation of a more reduced  $C_2$  compound. This action of oxygen can be considered to be an interaction of respiration and photosynthesis. It is too early to judge whether this may be looked upon as a competition of oxygen and carbon dioxide for certain hydrogen donors, although this may well be the case. Weigl *et al.* (1951) found not only that light decreased the respiratory evolution of carbon dioxide but also that intermediates from  $C^{14}O_2$  were not respired while the light was on but were as soon as the light was turned off. In a sense this is in harmony with the earlier statements of Brown *et al.* (1948) concerning some respiration-stable special photosynthetic intermediate. In the whole of knowledge now gained and in accordance with the present general concept of photosynthesis as outlined in Sect. 2, it is more plausible to look upon this again as a competitive activity of oxygen vs. carbon dioxide for these intermediates. The question arises, of course, as to why this competition is different in the light and in darkness if the same substances are involved. The preliminary answer would seem to be that

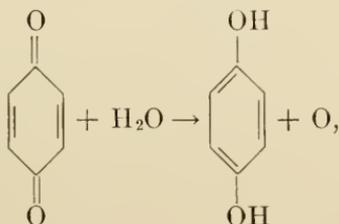
the continuous supply of reducing power by the action of the light and the "tendency to eliminate oxygen and oxidizing products" cause a shift of the redox potential, which promotes another type of metabolism also in parts of the system not directly affected by light. In this respect direct observations on the connection between photosynthesis and redox potentials (see later) are of interest.

All results obtained so far by using carbon isotopes in the study of photosynthesis fit very well into the general concept developed in the preceding sections. The chain of photosynthesis appears to consist in a transfer of hydrogen atoms (or eventually of electrons, or of "reducing power") from an ultimate hydrogen donor (water in the case of green-plant cells) to an ultimate acceptor (carbon dioxide). One of the links in this chain, obviously the basic or most important one—whether it is one single step or a more complex mechanism—requires light energy in order to work.

The present writer and his collaborators concluded: "We may visualize that, in the dark, the various links of the process are already being prepared with the aid of energy available in the structural elements of the cell. The role of the light energy is to make a chain of these links" (Dorrestein *et al.*, 1942, p. 367).

#### 4-4. EXPERIMENTS ON ISOLATED CHLOROPLASTS

The study of isolated chloroplasts, which is discussed in Chap. 6, is only briefly considered here in its bearing on the general mechanism of photosynthesis. Isolated systems such as chloroplasts and grana have so far been found incapable of photochemical carbon dioxide reduction, but with a variety of hydrogen acceptors other than carbon dioxide, a photochemical evolution of oxygen may occur (Holt and French, 1949). Only the very simple reaction discovered by Warburg and Lüttgens (1944),

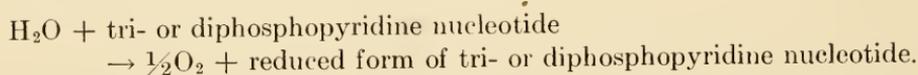


will be mentioned here.

At first the view was held that the process occurring in the illuminated chloroplast preparations was a direct photochemical splitting of water attached to the photosynthetic apparatus. Some observations, however, such as light-saturation phenomena (Holt and French, 1949), which indicate the participation of at least one dark reaction, afford evidence that a more complicated mechanism is in play. Warburg and Lüttgens (1944),

moreover, found evidence for the participation of a zinc-containing catalyst. It would seem probable that the light energy enters into a redox system of unknown composition (Wassink, 1947) which may be active in accepting energy from the pigment system. The acceptance of energy leads ultimately to the evolution of oxygen. The redox system might be restored in a dark process, supplying it with water or with hydrogen derived from water in a dark reaction. Holt and French (1949, p. 278), using water enriched with  $O^{18}$ , showed that the oxygen evolved by chloroplast preparations comes from water. In terms of the mechanism of photosynthesis, given in Sect. 2, the preparations appear to contain the systems (1) and (2), whereas system (3) is damaged or, at least, its connection with the first-mentioned systems is broken.

Vishniac and Ochoa (1951) recently succeeded in reestablishing in grana preparations a connection between the system of photochemical oxygen evolution and an enzymatic system for carbon dioxide fixation. The importance of this fact is emphasized by the circumstance that the coupling was achieved by the reversible oxidation-reduction of tri- or diphosphopyridine nucleotide. The authors summarize their findings by stating that illuminated chloroplasts are able to catalyze the over-all reaction



They furthermore suggest that chemosynthetic bacteria [cf. the studies by Vogler and Umbreit (1942) on *Thiobacillus thiooxidans*] may couple the oxidation of the specific substrate to the carbon dioxide fixation through the mediation of pyridine nucleotides. These observations and considerations are in accord with the views outlined previously in this chapter and advocated earlier by the present writer and his collaborators (Dorrestein *et al.*, 1942; Wassink, 1947; Wassink *et al.*, 1942) and also with observations on the connection between phosphate metabolism and photosynthesis, to be discussed later.

In view of the reduction of various hydrogen acceptors by illuminated chloroplast suspensions, it is of importance that intact algae may carry out similar reactions when reducible materials other than carbon dioxide are supplied. Fan *et al.* (1943) made experiments along this line. The following considerations, on which their work was based, are of interest in our discussion in view of the scheme proposed in Sect. 2: (1) oxygen liberated in photosynthesis originates from water; (2) some algae are capable of substituting oxidation of a hydrogen donor during photochemical carbon dioxide reduction for the oxygen evolution;<sup>3</sup> (3) isolated

<sup>3</sup> They mention Gaffron's (1944) experiments with *Scenedesmus* in which molecular hydrogen was found to act as a hydrogen donor. Another example of the same type is the oxidation of hydrogen sulfide during photosynthesis of some blue algae (Nakamura, 1938).

chloroplasts produce oxygen without simultaneous reduction of carbon dioxide. From these facts they concluded that carbon dioxide fixation and oxygen liberation are only "loosely connected"; this was the reason for their attempt to obtain oxygen from illuminated *Chlorella* cells by supplying reducible materials other than carbon dioxide. They succeeded in obtaining oxygen evolution with a variety of reducible substances, including ferric salts, acetaldehyde, benzaldehyde, and nitrourea. The following were among those which did not yield oxygen: potassium nitrate, *p*-dimethylaminobenzaldehyde, formaldehyde, butyl aldehyde, cystine, methylene blue, urea, methyl urea, succinate, citrate, fumarate, acetate, lactate, malate, glucose, and hexose mono- and diphosphate. Their conclusion (p. 17) is of interest: "It is also evident that the reactions with which we are dealing are not the results of a simple reducing action of illuminated tissue since only a relatively small number of materials are capable of causing oxygen production and a slight change in their structure alters this reaction." The reaction with benzaldehyde was studied in greater detail. No intermediate formation of carbon dioxide was involved. The chief conversion appeared to be:



The experiments were complicated by reactions causing disappearance of benzaldehyde in the dark.

Clendenning and Ehrmantraut (1950) compared photosynthesis with Hill reactions—the photochemical oxygen evolution from oxidizers other than carbon dioxide—in entire *Chlorella* cells in continuous and flashing light. An interesting observation is that the ability to photosynthesize in Warburg's carbonate-bicarbonate buffer No. 9 is irreversibly lost when the cells are exposed to a quinone solution in either light or dark. The maximum rate of oxygen production at light saturation was about the same with quinone as with carbon dioxide. Light saturation in the quinone reaction required, however, higher intensities. In flashing light it was found that the time required for completion of the limiting dark reaction was 0.03–0.04 sec at 10°C for both processes. The authors concluded that in both processes the same dark reaction, which thus cannot be concerned directly with carbon dioxide assimilation, enters as the rate-limiting process. In carbon dioxide assimilation proper, much slower reactions seem to be involved, as is evident from the work of Calvin *et al.* (see Calvin, 1949), showing that, after admission of  $\text{C}^{14}\text{O}_2$  for a few seconds during stationary photosynthesis, the  $\text{C}^{14}$  still is present chiefly in a "first product." Clendenning and Ehrmantraut (1950) also refer to work by Rieke and Gaffron (1943), who found that the time required to complete the limiting dark reaction in flashing-light experiments is the same for photosynthesis and photoreduction of hydrogen-adapted algae. This would rule out a reaction directly involved in oxygen liberation.

Clendenning and Ehrmantraut (1950) concluded that in their flashing-light experiments the limiting catalyst must be assigned to a reaction more closely connected with "the basic transformation of light energy into chemical energy, e.g., the stabilizing reaction of Franck and Herzfeld's catalyst B, or the energy-transferring reaction involving an intermediate acceptor, as postulated by Dorrestein, Wassink, and Katz." These studies, at least, clearly support the view already arrived at, namely, that dark processes are involved in the photochemical evolution of oxygen from quinone. An additional interesting observation made was that the Hill reaction showed no induction phenomena, irrespective of the oxidant. Franck and Gaffron (1941) have ascribed the induction phenomena to the presence of a natural narcotic. Clendenning and Ehrmantraut (1950) were led to stress another view, namely, that a limiting factor, e.g., lack of "carbon dioxide acceptor" after a dark period and its gradual synthesis in light, might be the cause of the induction phenomena. This would be in accordance with Calvin and Benson's views (1948), with the recent conclusions reached by Fager *et al.* (1950), and with the views reached by Wassink and coworkers from their combined studies of photosynthesis and pigment fluorescence in algae and in purple sulfur bacteria (see Wassink and Katz, 1939; Wassink *et al.*, 1942; Dorrestein *et al.*, 1942; Wassink and Kersten, 1943-1945). In an extensive discussion of the available kinetic work from various sources on chlorophyll fluorescence and photosynthesis, Wassink (1951b) arrived at the conclusion that Franck's views (1949) would be brought much nearer to his own when the hypothetical "natural narcotics," which are postulated to be produced in part by fermentation and in part by oxidation, were replaced by the postulate of a "limiting factor."

#### 4-5. THE CONNECTION BETWEEN REDOX POTENTIALS AND PHOTOSYNTHESIS

The general concept of photosynthesis to which the combined studies of fluorescence and gas exchange (Dorrestein *et al.*, 1942) have led made it worthwhile to investigate this connection. If a suspension of purple sulfur bacteria, *Chromatium*, is illuminated, distinct shifts in the redox potential are revealed if platinum electrodes in the suspension are brought into connection with a reference electrode. It is interesting that these shifts were shown to have a definite bearing upon the prevailing conditions for photosynthesis (Fig. 5-15). Suspensions of *Chromatium* are illuminated in phosphate buffer at pH 6.6 in  $N_2 + H_2$ , in  $N_2 + H_2 + CO_2$ , and in  $N_2 + CO_2$ , respectively. With the start of the illumination, a potential shift toward the oxidized side is observed in all cases; it is different, however, in magnitude, depending on the special conditions (Wassink, 1947). It is smallest in  $N_2 + H_2$ , somewhat larger in  $N_2 + H_2 + CO_2$ , and largest in  $N_2 + CO_2$ . Gas exchange is negligibly small

in the first and last and is very high in the second case. When the light is shut off, the potential quickly falls almost to the initial value. Obviously no relation exists between the over-all gas exchange and the potential shift. This means that the potential shift is not due to final products or to waste products from the over-all process. The conclusion seems justified that the potential change is related to a shift in the state of oxidation-reduction of an essential intermediate catalyst; this shift is due to the interference of the light.

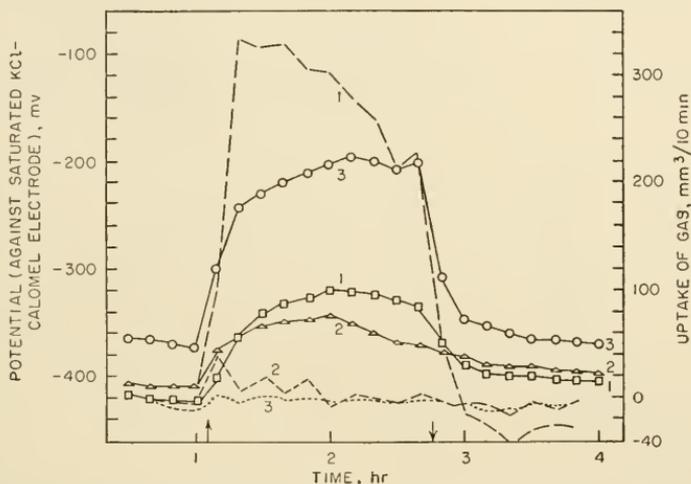


FIG. 5-15. Redox potential and gas exchange in suspensions of *Chromatium*, strain D. Solid lines: potentials; broken lines: uptake of gas. Curves No. 1:  $N_2 + 30\% H_2 + 5\% CO_2$ ; No. 2:  $N_2 + 15\% H_2$ ; No. 3:  $N_2 + 5\% CO_2$ .  $\uparrow$ light on;  $\downarrow$ light off. Reference electrode: saturated KCl-calomel electrode; suspension medium: phosphate buffer, pH 6.6,  $29^\circ C$ . (From Wassink, 1947.)

The chain of photosynthesis is essentially a hydrogen-transfer chain. In the absence of hydrogen and with excess carbon dioxide, the intermediate reversible redox systems will be readily dehydrogenated, causing a shift of their stationary potential to the oxidized side. It seems logical to assume that, in the beginning, intermediate products and reduced substances will be able to supply some hydrogen but that this source will quickly become exhausted. This is in perfect agreement with the observation that in  $N_2 + CO_2$  the potential, after a quick, steep rise immediately after the start of the illumination, subsequently shows a slower, gradual rise for a long time (Fig. 5-15). On the contrary, with nitrogen + hydrogen the potential does not rise much above the dark value and, moreover, tends to decrease during illumination, so that, when the illumination is discontinued, hardly any change is observed. This indicates that initially some hydrogen acceptors still are available, but they gradually become exhausted, so that the intermediate redox systems shift more to the reduced side. Perhaps the initial rise is due chiefly to carbon diox-

ide or to oxidized products formed in the dark period in the beginning of the experiment.

It was tempting to try to obtain evidence for the generation of a reducing power also from direct potential measurements. Wassink (1949) and Wassink and Kuiper (in preparation) obtained a distinct potential shift to the reduced side only when suspensions of *Chromatium* were illuminated in  $N_2 + 30$  per cent  $H_2$  at a pH of about 8.0 (Fig. 5-16). Under these conditions the uptake of gas that could not be suppressed by the presence of potassium hydroxide and thus was taken to be hydrogen was

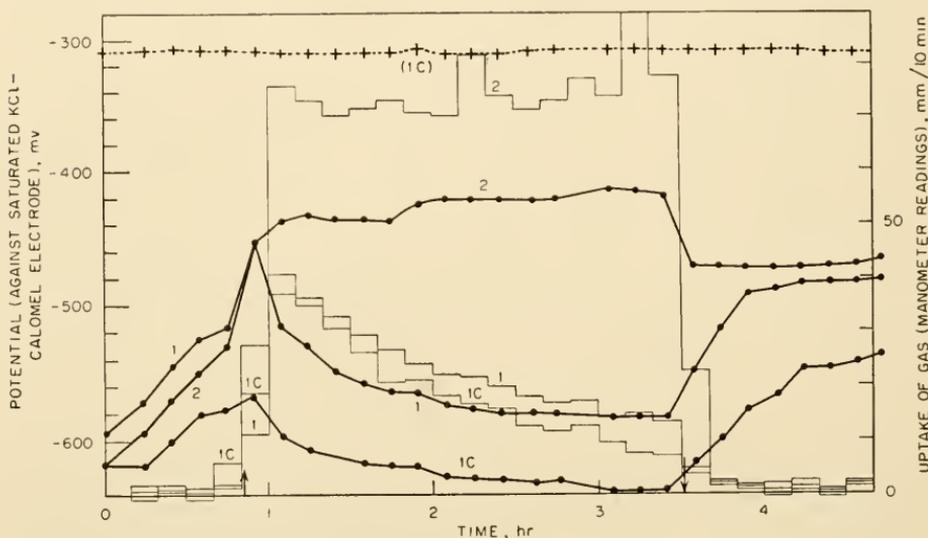


FIG. 5-16. The same as Fig. 15, but at pH 8.0. Heavy lines: potentials; light lines: gas exchange. Curves No. 1:  $N_2 + 30\%$   $H_2$ ; No. 1C: the same, but in the presence of KOH, (1C) glass electrode record in the same vessel; No. 2:  $N_2 + 30\%$   $H_2 + 5\%$   $CO_2$ . (From Wassink and Kuiper, in preparation.)

much higher and more resistant than at a lower pH, at which initially only a small uptake of gas was observed. In the beginning of the illumination a potential shift to the oxidized side is often observed which seems due to the presence of some carbon dioxide produced in the previous dark period.

Because the combined measurements of fluorescence and photosynthesis had shown that cyanide inhibits preferably the carbon dioxide side of the reaction chain, it was tempting to try to demonstrate directly the reducing effect of light under conditions of cyanide inhibition. It turned out, however, that in the presence of cyanide the potential shift to the reduced side (in  $N_2 + H_2$ ) was converted into a shift to the oxidized side, so that no appreciable difference existed with the curve obtained in  $N_2 + H_2 + CO_2$ . It was concluded that a hydrogenase that is especially susceptible to cyanide is activated by light (at pH 8.0). This theory

of activation of a hydrogenase in light is in agreement with the development of a reducing action by the light. The following observation, made by Gaffron (1944), is probably related to the one just mentioned. Cells of *Scenedesmus*, "adapted" to hydrogen, show a photochemical evolution of hydrogen. In a nitrogen atmosphere these cells produce hydrogen also in the dark, but the rate is at least ten times higher in the light. Gaffron assumed that the photochemical evolution of hydrogen is dependent upon the presence of suitable hydrogen donors in the cells. The observed photoacceleration of a hydrogenase activity may well be considered as evidence for a direct reducing effect of the light.

A phenomenon that probably belongs to the same type is the evolution of hydrogen in the light by suspensions of the purple bacterium *Rhodospirillum*, as observed by Gest and Kamen (1949) and by Gest *et al.* (1950). Since it does not occur in the dark, it may again be considered as a sign of the generation of reducing power in the light. For the reduction of carbon dioxide, substrate hydrogen seems preferable to gaseous hydrogen. Hydrogen production is inhibited by molecular nitrogen and ammonium ions. It thus seems that nitrogen and ammonium compounds are preferable acceptors for this form of reducing power.

Gaffron (1937) once assumed that, at the beginning of a light period, a catalyst of the Blackman system is reactivated by conversion into a reduced state. In other cases, observations were made which suggest that the *over-all effect* of light in photosynthesis of green cells results in a shift to the oxidized side, just as was found in purple sulfur bacteria. Fluorescence, for example, shows a downward trend, pointing to an increased state of oxidation obviously related to the production of oxygen (Wassink and Katz, 1939). Also a few direct measurements of the redox potential in green-cell suspensions show a shift to the oxidized side during illumination (Tang and Lin, 1937). Brief illuminations of suspensions of *Chlorella* and of diatoms, i.e., during 5 or 10 sec each minute with interspersed dark periods, keep fluorescence at a high level, indicating the maintenance of a relatively reduced state. Under these conditions a dark system appears to be inactivated, which leads to an unchanged rate of oxygen production at low light intensities but decreased production at high intensities (cf. Wassink and Kersten, 1943-1945, and Fig. 5-17). At illuminations of 10 sec each minute, the effect is about intermediate between the behavior at 5 sec each minute and that with continuous illumination. The reactivation of the dark system proceeds along with oxygen production, and in connection with the fluorescence measurements it seems reasonable to assume that this catalyst is more active in an oxidized state. The reactivation of this dark catalyst, however, very likely is not a direct effect of light and does not interfere with the view that light produces a reducing power. It shows that in the stationary conditions of normal photosynthesis, with respect to the general

state of the cell, the reducing effect of the light is dominated by oxidative tendencies.

The work of Aronoff (1946a,b), who found that the reduction of quinones by chloroplast suspensions (see also Chap. 7) under certain conditions was related to the redox potential of the quinones (benzo-, naphtho-, and anthraquinone), will be briefly mentioned. To a certain extent this is the reverse experiment of that by Wassink (1947, 1949), who found that metabolism creates a definite shift in redox potential. Aronoff's experiments tend to show that a redox potential imposed from outside influences at least the rate of reaction. Some observations by Aronoff (1946a), however, suggest that this view may be too simple.

Gerritsen (1949-1950, 1951) made rather extensive measurements of redox potential and pH shifts in chloroplast preparations of *Avena* leaves upon illumination under various conditions. A strong shift of the potential to the oxidized side was observed upon illumination, as well as consumption of ascorbic acid. It seems conceivable that photooxidations play a more important role in the observed phenomena than do partial processes of photosynthesis. When a small amount of carbon dioxide is introduced, the suspension becomes more alkaline upon illumination, whereas the redox potential decreases considerably. From this Gerritsen (1951) concludes that carbon dioxide participates actively

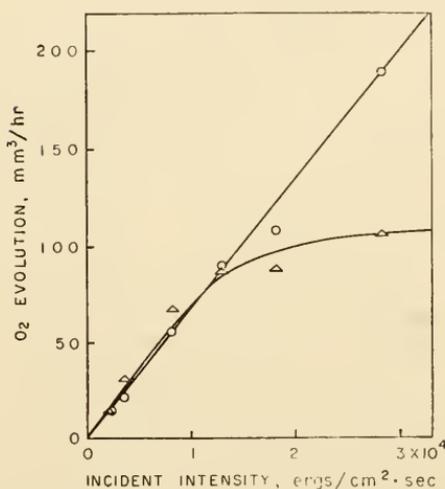


FIG. 5-17. Rate of photosynthesis vs. incident intensity in diatoms (*Nitzschia dissipata*) with repeated exposures of 5 sec/min ( $\Delta$ ) and under continuous light ( $\circ$ ) (Warburg buffer No. 9, 25°C). (From Wassink and Kersten, 1943-1945.)

in photochemical processes in anaerobic crude cell-free chloroplast (or grana) suspensions. He claims that in anaerobic suspensions carbon dioxide is hydrogenated photochemically by cell constituents of the type  $\text{RH}_2$ .

#### 4-6. ON THE CONNECTION BETWEEN PHOSPHATE METABOLISM AND PHOTOSYNTHESIS

Calvin *et al.* showed that the action of the "reducing agent" upon the carbon dioxide complex (see Sect. 2) first leads to the formation of 2- and 3-phosphoglyceric acid (see Sect. 4-3). Thus this first detectable compound of carbon dioxide assimilation contains phosphate. Also the carbon dioxide acceptor will therefore contain phosphate; it has been

suggested that it is vinyl phosphate (Benson and Calvin, 1950). It seems unlikely that this substance as such is identical with the "reducing power," but it may well be generated rather directly from this reducing power, i.e., in a reaction sequence:



in which FH eventually might represent the vinyl phosphate, EH the "energy acceptor" normally present at the pigment-protein complex, and DH the ultimate hydrogen donor (cf. also Wassink, 1947).

Vogler (1942) suggested that in photosynthesis radiant energy may be stored in a form available for CO<sub>2</sub> reduction, from analogy with his experiments on the metabolism of *Thiobacillus*. In view of the facts observed by Vogler and Umbreit (1942) on the coupling of the energy-producing sulfur oxidation in *Thiobacillus* with carbon dioxide assimilation by presumably energy-rich phosphate compounds, it is tempting to look upon these compounds as connected with the storage of radiant energy in photosynthesis also. In *Thiobacillus* inorganic phosphate is taken up during the oxidative phase and converted into an adenosine-triphosphate, whereas during the reductive phase phosphate is released (LePage and Umbreit, 1943).

A few observations on phosphate interactions with photosynthesis have since been made (Aronoff and Calvin, 1948; Emerson *et al.*, 1944), but not with very conclusive results. In the writer's opinion this could be due to the fact that no separation of an eventual phosphate-accumulating phase and a phosphate-consuming phase had been attempted. This was the basis of some studies by Wassink *et al.* (Wassink *et al.*, 1949; Wassink *et al.*, 1951), who illuminated suspensions of *Chromatium* and of *Chlorella* under various gas phases. In all cases the absence of carbon dioxide led to a markedly increased consumption of phosphate. In *Chlorella* it was shown that the phosphate taken up is converted into a TCA-insoluble form (cf. Fig. 5-18). These findings are in accordance with studies by Gest and Kamen (1948, p. 309), who, using tracer phosphate in *Rhodospirillum*, concluded that uptake and turnover are both much greater in the light than in the dark. Simonis and Gruber (1952) found P<sup>32</sup> phosphate uptake by *Helodea densa* increased by light and carbon dioxide. With the colorimetric technique used by Wassink *et al.*, the turnover of phosphate between fractions that maintain the same stationary concentrations cannot be detected. Under the conditions chosen, however, a shift results, the explanation of which goes along similar lines as that given for the redox-potential shifts.

Kandler (1950), working with *Chlorella*, measured short-time shifts of TCA-soluble phosphate and found distinct changes in the phosphate level (TCA-soluble) at the shift from dark to light and vice versa. Since

carbon dioxide was present, he saw no difference in the stationary level in light and darkness.

The role of the phosphate compounds in photosynthesis is not very clear from an energy point of view. Even if 10 quanta is allowed for the reduction of one molecule of carbon dioxide (see Chap. 4), in case of exclusive generation of phosphate bonds only about 120 kcal/mole would be available for the reactive systems, from which 112 kcal would have to be fixed as  $\text{CH}_2\text{O}$ . Even if the formation of two high-energy phos-

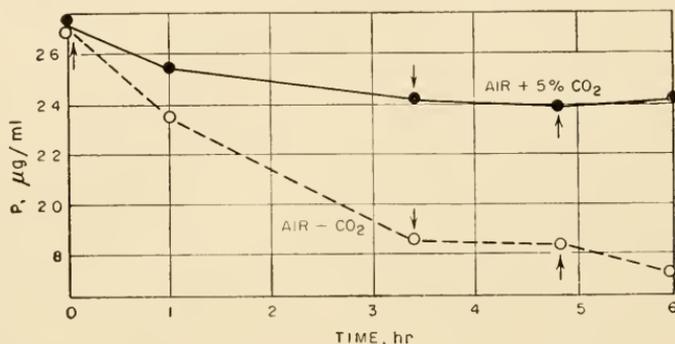


FIG. 5-18. Changes in trichloroacetic acid-soluble phosphate in suspensions of *Chlorella* in the presence or absence of  $\text{CO}_2$  (pH  $\sim$  4.0, 25°C).  $\uparrow$  light on;  $\downarrow$  light off. (From Wassink *et al.*, 1951.)

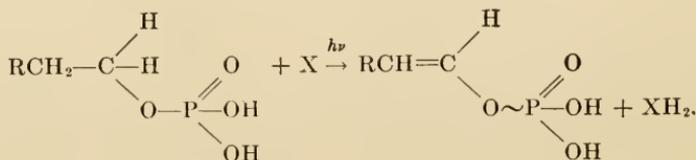
phate bonds per quantum were accepted, the space for energy losses would not be too large, especially since some authors accept a lower quantum number (see, for example, Kok, 1948; Warburg *et al.*, 1950). Recently Wassink (1951c) suggested that in connection with the phosphate cycle an active form of hydrogen might also be generated.<sup>4</sup> A similar view is discussed in some more detail by Kandler (1950), with some interesting estimations of energy. Recently Holzer (1951) proposed a scheme of photosynthesis in which a role was attributed to ATP.

The evidence so far obtained shows that at least the transfer of light energy [system (2), Sect. 2] is intimately connected with phosphate conversions.

### 5. SOME REMARKS ON ENERGY TRANSFER IN PHOTOSYNTHESIS

In this section a few remarks on energy transfer may be added. This may be considered as a discussion of part of process (2), given in Sect. 2.

<sup>4</sup> This view was expressed at a Symposium for Biokatalysis at Utrecht, Feb. 2, 1951. Professor E. Havinga, Leiden, then suggested the following possibility [cf. Wassink (1951c), p. 977]:



It is clear that, in some way or other, in photosynthesis amounts of energy are being translocated which have their origin in the light energy. Some possible modes of energy transfer will be discussed here. The transport of energy in a stage of the process not far apart from light absorption first became clear from considerations by Gaffron and Wohl (1936) in a combined discussion of the results of quantum measurements by Warburg and Negelein (1923) and of experiments in flashing light by Emerson and Arnold (1932). Gaffron and Wohl (1936) concluded that the high energy yield found in Warburg's experiments is comprehensible only if some 1000 chlorophyll molecules are able to provide energy for a single "reducing centrum." Gaffron and Wohl accepted the existence of "photosynthetic units" containing 2000-3000 chlorophyll molecules. The further history of this concept and its criticism cannot be followed here in detail. The original concept of the unit strongly adhered to the view that a carbon dioxide complex acts as an energy acceptor and has to receive 4 quanta within a short time in order to be reduced to carbohydrate. If the view is accepted that a relatively stable reducing agent is generated with each separate quantum—for this the observations on uptake of carbon dioxide after illumination and those on accumulation of TCA-insoluble phosphate compounds furnish evidence—the special difficulties for which the original concept was set up no longer exist. As soon as a sufficient concentration of activated energy acceptor (or reducing agent) is built up, each carbon dioxide molecule will be able to meet a sufficient number of acceptor molecules. The experiments leave sufficient room for the postulated building of such a concentration, since the rate of photosynthesis is stationary only after some minutes, during which fairly reproducible changes occur [induction phenomena; see van der Veen (1949-1950); Wassink and Katz (1939); Wassink and Kersten (1943-1945)].

The transport of a reducing agent, from the viewpoint of the metabolizing cell, is an energy transport with a material bearer (an electron, a radical, or a molecule). It would be covered by the "energy transport by diffusion" of Möglich *et al.* (1942). Möglich also distinguishes various other types of energy transport, some of which may have a bearing on a more direct energy transport within the chlorophyll-protein complex [chlorophyllin (Wassink, 1948b)] or between the adjacent pigment molecules themselves.

Evidence for such an energy transport is especially furnished by the fluorescence of chlorophyll resulting from light absorption by other pigments (e.g., fucoxanthin or phycoerythrin; cf. Sect. 3). In the case of fucoxanthin the yield of both photosynthesis and chlorophyll a fluorescence was the same as for light absorbed by chlorophyll a itself. Fucoxanthin obviously is bound to protein (cf. Sect. 3). The possible modes of energy transport in the chromophyllin are illustrated in Fig. 5-19, upper

row. In the case of pigment-protein complexes with chlorophyll a and b, a choice between these possibilities could be made, as the writer (1948a) pointed out, by studying fluorescence spectra upon irradiation with light of wave lengths absorbed preferably by either chlorophyll b or a (cf. Fig. 5-19). Duysens (1951) meanwhile carried out such experiments and found a 100 per cent transfer of energy from chlorophyll b to a, i.e., from the higher excitation (shorter wave length) to the lower. This rules out the third possibility for the chlorophyll a-chlorophyll b chromophyllins (see Fig. 5-19). In the case of the fucoxanthin-chlorophyll a

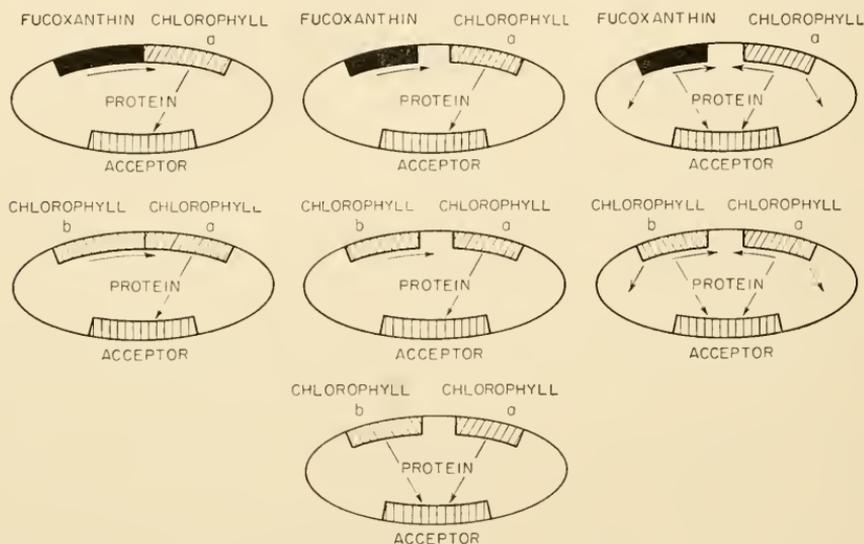


FIG. 5-19. Possible modes of energy transfer inside the fucoxanthin-chlorophyll a chromophyllin of diatoms and inside the chlorophyllin of green plants. (From Wassink, 1948b.)

chromophyllins this cannot be decided, since fucoxanthin does not show fluorescence. By analogy this now seems less probable than the one-sided transfer. It could, however, not be excluded beforehand, since, according to Möglich *et al.* (1942), the type of energy transfer designated as "energy transport with the cooperation of various degrees of freedom" may be especially important in large complexes. Such complexes might provide an energy supply from their thermal motion to enable an energy transport toward a higher level. So far as the evidence now goes, however, this mode of transport does not seem to be very important in photosynthesis. It should be borne in mind, however, that in living cells thermal motion may be influenced by other sources of energy production.

Whether transfer of energy from one pigment of a chromophyllin to another goes directly or via the protein cannot now be decided. Both paths seem conceivable. Förster (1947) discussed the theoretical possibilities of transport of energy from one chlorophyll molecule to another

in a solution. He concluded that for chlorophyll a solutions, with a mean distance between neighboring molecules of 80 Å, transfer of energy has the same probability as annihilation. The concentration for which this holds is about a hundred times smaller than that in the chloroplasts. This would mean that energy transfer over  $10^4$  molecules is feasible. Förster (1947, p. 177) considers the way of transport of energy as an "exciton" that goes from one molecule to another like a material corpuscle in three-dimensional Brownian movement. It follows from his computations that this type of energy transport would permit rather large-distance transport of energy in the chloroplasts. It does not, however, exclude energy transport through the protein. In photosynthesis this has not yet been directly demonstrated, but for photolysis of carbon monoxide myoglobin, Bücher and Kaspers (1946, 1947) have demonstrated that light absorbed by the protein has the same efficiency as that absorbed by the pigment (Fig. 5-20).

The problem is to find out which of the various possible ways of energy transport actually is followed. Perhaps there are different ones operating simultaneously, and it is tempting to conclude this discussion with the quotation of a view, expressed by Engelmann in 1883: ". . . dass man es innerhalb jedes Chromophyllkörpers mit einer molekularen Mischung zu thun (hat), d.h. die Farbstofftheilchen sind so innig und gleichmässig mit den farblosen Stromatheilchen des Chromophyllkörpers vermengt, dass allerorts zwischen beiden Molekularwirkungen stattfinden können."

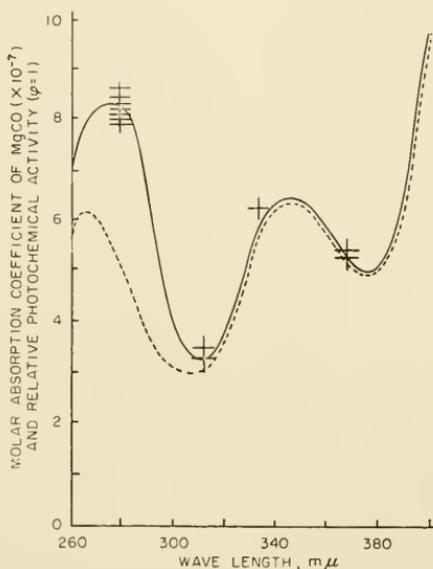


FIG. 5-20. Absorption spectrum of CO myoglobin (solid line) and of its hemin component (dotted line), and relative photochemical activity of some wave lengths in splitting off CO (crosses). (From Bücher and Kaspers, 1946.)

## SUMMARY

Photosynthesis is essentially a light-sensitized hydrogen transfer from a hydrogen donor to carbon dioxide. It seems advisable to specify photosynthesis as carbon dioxide photosynthesis, since experiments both with cell-free pigment-protein preparations and with intact cells have demonstrated that carbon dioxide may be replaced by certain other substances. The ultimate hydrogen donor for green-plant-cell photosynthesis is water,

but that for the purple bacteria is a variety of more reduced substances (Sects. 1 and 2). Evidence has accumulated in favor of the view that the photosynthetic chain may be divided into three well-distinguishable parts, each of which may consist of a number of elementary steps. The three parts are (1) the dehydrogenation of a hydrogen donor, (2) the "photochemical process" proper, and (3) the reduction of carbon dioxide (Sect. 2.) The light energy enters directly only into link 2; the other processes consist of only dark reactions. The first noticeable chemical act of the light is the production of a "reducing agent." The evidence for this view has been collected from various independent lines of research in relation to photosynthesis, e.g., studies on comparative biochemistry, studies of chlorophyll fluorescence along with photosynthesis, studies using tracer elements (e.g.,  $C^{14}$ ), *in vitro* studies of partial reactions, studies on redox potentials, and studies on phosphate conversions. A detailed discussion of the evidence from these various fields is given in Sect. 4.

Some remarks on the structure of the photosynthetic apparatus are given in Sect. 3; some remarks on possible modes of energy transfer inside the chloroplasts or photosynthetic bacterial cells are given in Sect. 5.

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#### ADDENDUM\*

Notwithstanding the long time elapsed since this chapter was written, it still would seem to represent a fairly adequate general outline of the field. Nevertheless, the author feels that it is useful to add a few references, in which specific progress is either communicated or surveyed. The content of most of them is clear from their titles; if not, a brief comment is added. The selection, of course, is arbitrary, but an attempt has been made to adapt it more or less to the outline given in the chapter.

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\* Added in proof, March, 1955.

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*Photosynthesis of algae, with a view to mass-culturing*

- Burlew, J. S. (ed.) (1953) Algal culture from laboratory to pilot plant. Carnegie Inst. of Washington, publ. 600, Washington, D.C.
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## CHAPTER 6

# The Absorption, Action, and Fluorescence Spectra of Photosynthetic Pigments in Living Cells and in Solutions<sup>1</sup>

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*Introduction. Absorption spectra: Measurement of absorption spectra in scattering media—Absorption and reflection spectra of leaves and algae—Absorption spectra of some purified chloroplast pigments. Action spectra of photosynthesis. Fluorescence spectra of photosynthetic pigments: Fluorescence spectra of the extracted pigments—Transfer of energy between pigments in live plants as determined by fluorescence spectroscopy. Spectra of photosynthetic bacteria: Absorption spectra of photosynthetic bacteria and of their pigments—Action spectra of photosynthesis, phototaxis, and fluorescence excitation in purple bacteria. Concluding remarks. References.*

### INTRODUCTION

Most of the pigments of plants that live by photosynthesis have been extracted by organic solvents, then purified and crystallized. Their chemical compositions and reactions are well known, their absorption spectra have been precisely measured, and the photochemical reactions of the most important plant pigment, chlorophyll, have been investigated in solutions of the pure substance. There is, however, a striking contrast between the detailed knowledge of the behavior of the isolated pigments and the great lack of information available on the properties and reactions of these same pigments as they occur and function in living plants. A simple comparison of the absorption spectra of pure chlorophyll solutions with the absorption spectra of leaves or of algae shows at once that in the living system, capable of carrying on photosynthesis, the pigments are very different. The failure of all attempts to reproduce a photosynthetic system with purified pigments appears to be in part due to the different nature of the intact and the extracted pigments.

The chemical information about the purified pigments is of great value in identifying and measuring the quantity of the various pigments

<sup>1</sup> Manuscript submitted for publication Dec. 14, 1951; only a few references were added later, although the field has developed appreciably since then.

obtained from plants. The process of photosynthesis and the function of the pigments must still be studied in the living material.

There are three types of spectroscopic data that are used to determine the identity, quantity, and function of photochemically active pigments. Absorption spectra of live cells can be used to detect the presence of certain pigments, but only very crudely to determine the quantity present. They also give information as to the fraction of the incident light absorbed by cells. Quantitative measurements of spectral absorption are most useful in work on the nature and concentration of pigments in solutions outside the cell. Fluorescence spectra both of solutions and of live cells provide an equally powerful method of identification and provide, furthermore, the only direct measure of the energy received by the various individual pigments when a complex mixture such as exists in biological material is illuminated. Action, or effectiveness, spectra show what regions of the spectrum cause a photochemical or photobiological reaction to take place. Comparisons of action spectra with the absorption spectra of known compounds are used to identify the light-absorbing pigment for the reaction in question.

It is the purpose of this chapter to present on uniform scales the various types of spectroscopic measurements that are often needed by workers in the field, to summarize the main findings from spectroscopic measurements of photosynthetic plants and of their isolated pigments, to show the type of information that can be obtained by biological spectroscopy, and to point out some of the more obvious gaps in the knowledge of the spectroscopy of the photosynthetic pigments in live cells. The present discussion has been restricted to the consideration of complete spectral curves and intentionally omits all data reported only as wave length of absorption, fluorescence, or effectiveness maxima.

The spectroscopy of photosynthetic pigments has very recently been given a comprehensive and critical treatment by Rabinowitch (1951), where many of the subjects are covered in more detail than can be done here. Algal pigments have been reviewed by Cook (1945).

By measurements of action spectra it has been found that light absorption by at least 10 pigments can induce photosynthesis—namely, chlorophyll a, chlorophyll b, bacteriochlorophyll, bacterioviridin, fucoxanthin, some bacterial carotenoids, C-phycoerythrin, R-phycoerythrin, C-phycoerythrin, and R-phycoerythrin. Of these pigments, chlorophyll a is the only one that has been found in all photosynthesizing plants, with the exception of bacteria that have one of two pigments, bacteriochlorophyll or bacterioviridin, closely related to chlorophyll a. The universal occurrence of chlorophyll in organisms capable of photosynthesis has led observers to wonder whether it is the only pigment that is essential for the process. If so, perhaps the other pigments act merely by transferring their absorbed energy to chlorophyll. The answer to this question has

been sought by investigating the ability of light absorbed by these other pigments to excite the fluorescence of chlorophyll. This involves the determination of effectiveness spectra for the excitation of chlorophyll fluorescence in various organisms and the correlation of these spectra with the absorption spectra of the individual pigments. Experiments of this type have indeed shown that energy transfer takes place from accessory pigments to chlorophyll. This subject was reviewed recently by Smith (1949), but new publications have appeared since then, permitting a more quantitative treatment of results. Much of the research on action spectra has yielded results which are not sufficiently precise to permit quantitative handling of data and which have consequently been omitted from this article. One of the main technical weaknesses has been the use of light that was not sufficiently monochromatic. There are a number of physical and biological sources of errors that may enter into the interpretation of action spectra in living organisms. These have been discussed by Blum (1950). Factors that have hindered the interpretation of data on photosynthesis are (1) difficulty in estimating the true absorption, corrected for scattering of light, in living organisms; (2) unavailability of absorption spectra for the individual components *in vivo*; (3) difficulty in estimating the internal filtering effect of light by non-participating components that absorb selectively with respect to wave length; and (4) inadequate knowledge concerning the dependence of quantum yield upon wave length for the individual pigments.

The best available data have been interpreted in view of these limitations and have been presented here to show the correlations that have been made between action, absorption, and fluorescence spectra in various photosynthetic organisms. The means by which these correlations have been made are presented to show how these procedures may be applied to other biological processes in the study of mechanisms of various photobiological effects. Owing to the fundamental importance and wide distribution of the chloroplast pigments, the correlation between effectiveness and the absorption spectra of their pigments has been worked out in some detail.

Since consideration of action spectra must be prefaced by a knowledge of the absorption spectra of living organisms and also of extracted pigments, this information is presented first.

Because the photosynthetic process and the pigments of bacteria are different in some major respects from those of higher plants, the bacteria have been treated in a separate section at the end of the chapter.

*Graphical Presentation of Spectroscopic Data.* Often for very good reasons the same type of data is presented by different authors in very different ways. Thus measurements of the absorption spectra of solutions may be plotted with abscissas of wave-length units, wave-number units, frequency units, or electron volts. The ordinates of these curves

may be given as percentage of absorption, optical density, or the logarithm of the optical density. These various scales have, for certain purposes, different degrees of usefulness. Any comprehensive intercomparison of data requires, however, that the scales be identical. We have therefore replotted all the curves presented in this paper on the same wavelength. Even with uniformity as an objective, it has not been practical to use the same scale throughout this article. For most purposes optical-density scales are preferable, but for comparison with action spectra per cent absorption curves are generally used. Many curves originally given as percentage of transmission or as logarithm of density have been converted to optical density, and in most cases the optical density of live material at the position of the chlorophyll peak in the neighborhood of 678  $m\mu$  has been adjusted to 0.6, thus making possible a direct comparison between many different curves by simple tracing. These transformations have been carried out by use of a graphical computing machine (French *et al.*, 1954). Many absorption spectra are published with much too short a wave-length scale and an exaggerated height. These may have a dramatic appearance but are nearly impossible to use in finding the absorption coefficient for a particular wave length.

## ABSORPTION SPECTRA

### MEASUREMENT OF ABSORPTION SPECTRA IN SCATTERING MEDIA

The measurement of true pigment absorption in leaves or suspensions of algae which, in addition to absorbing, also scatter large amounts of light in all directions is indeed difficult. Many investigators have taken up the problem of making such measurements in leaves. We have here omitted reference to all work without scattering corrections, work done with broad spectral regions, or data including too few wave lengths to give complete spectra. The papers by Seybold and Weissweiler (1942a,b) are much more extensive than any of the previous work and review the absorption-spectrum measurements of many other workers. Confirmatory data and a small extension of the wave-length range toward the near infrared are given by Rabideau *et al.* (1946). More recent absorption and reflection data are given by Moss and Loomis (1952). Specific work on the absorption of particular algae will be referred to later in connection with their action spectra.

Taking the absorption of light by a clear homogeneous solution after correction for specular reflection of light at the surface of the vessel, we have the relation  $A + T = 1$ , where the incident light is set equal to 1;  $A$  and  $T$  are, respectively, the absorption and transmission of the solution concerned. The transmission varies with the thickness  $l$  of the layer, the concentration  $c$  of the absorbing material, and the absorption coefficient  $\alpha$  according to Beer's law:

$$D = \alpha lc = \log \frac{I_0}{I} = \log \frac{l}{\bar{l}} = \log \frac{1}{1 - A}$$

In this equation the optical density  $D$  bears a logarithmic relation to the reciprocal of the transmitted light,  $I_0$  is the incident light, and  $I$  is the transmitted light.  $D$  is directly proportional both to the length of the light path within the solution and to the concentration of the absorbing material. In heterogeneous systems, such as leaves or algae that scatter as well as absorb, these relations no longer apply strictly. The non-specular forward scattering of light, also termed "reflection," may be of considerable significance. Furthermore, if a heterogeneous object scatters light, the optical path length within it is no longer equal to the thickness of the object, and it is not readily measurable. If, as in chloroplasts, the pigment is aggregated within grana, with clear spaces between the grana, the concentration of pigment also becomes indeterminate for optical purposes. The transmission of clear solutions is ordinarily measured in spectrophotometers, which pass a narrow beam of monochromatic light through the solution and then to a photocell. Most spectrophotometers that do not depend upon the use of an integrating sphere, such as that in the Hardy recording spectrophotometer, are not suitable for measurements of light absorption by systems that scatter light, since the photocells are ordinarily too far removed from the vessel to catch much of the diffused light. Measurements of transmitted light may be made fairly well by the use of a large photovoltaic cell or a piece of opal glass placed directly behind the leaf or cell suspension. Such a method has been used by Emerson and Lewis (1943) in measurements on *Chlorella* to be discussed later in this chapter, and also by Chen (1951) on chloroplasts. This procedure does not, however, take into account the light scattered and reflected from the front surface. Measurements of forward and side scattering, as well as transmission of bacterial suspensions in glass boxes of different sizes, are given by French (1937a). These data have also been used to evaluate the deviations from Beer's law in scattering suspensions. An integrating sphere of the type ordinarily used in making measurements of the total output of incandescent lamps is inherently free of errors due to light scattering. This has been applied by Seybold and Weissweiler (1942a,b) and by Rabideau *et al.* (1946) to measurements of leaf absorption. The principles involved in sphere measurements have been described in some detail by Kok (1948). Figure 6-1 shows some of the different ways in which integrating spheres may be used for this purpose. The elegantly simple arrangement of Haxo and Blinks (1950) requires a correction for the light reflected from the surface of the photonic cell and absorbed by its second passage through the algae (C. Yocum, personal communication, 1951).

Rabinowitch (1951) reviews some means that have been used for

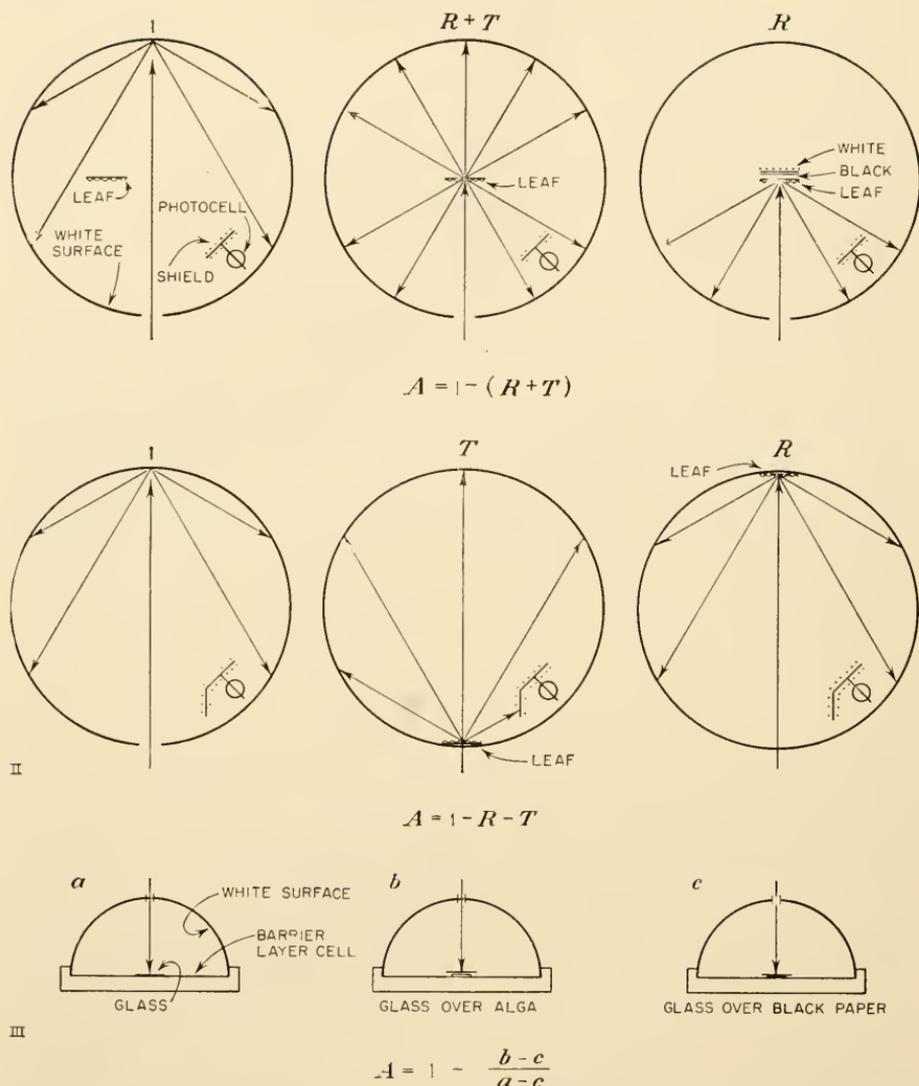


FIG. 6-1. The measurement of absorption, reflection, and transmission of a leaf or algal suspension by the integrating sphere. (I) Procedure of Rabideau *et al.* (1946). (II) Procedure of Seybold and Weissweiler (1942a) using the Hardy recording spectrophotometer. (III) Haxo and Blinks (1950) half-sphere method.

correcting absorption data in scattering media for errors of path length which are not taken care of by integrating-sphere measurements. It is, however, difficult to see just how such corrections can be used with the data available.

#### ABSORPTION AND REFLECTION SPECTRA OF LEAVES AND ALGAE

The study of absorption spectra of leaves and algae has been of interest for various reasons: (1) to determine how light absorption by various

plants affects their distribution in nature, (2) to determine the efficiency of light utilization in photosynthesis, (3) to determine which pigments are present in various organisms, (4) to attempt to characterize the physical and chemical state of the pigments in living organisms, (5) to measure the concentrations of pigments, (6) to make comparisons with action spectra in order to find which pigments utilize the light they absorb for photobiological processes, (7) to provide information for the art of camouflage. We will emphasize particularly those absorption spectra which relate to the interpretation of action spectra. Inasmuch as the absorption spectra of the individual components *in vivo* are only very crudely measurable, the absorption spectra of the pigments in organic solvents are of great value for this purpose.

One of the earlier reasons for interest in the absorption spectra of leaves and of algae was primarily ecological. The questions from this point of view are: How efficient are leaves as light traps? Does the efficiency of light absorption by a leaf account for the death or survival of a plant in nature under light-limiting conditions? For ecological purposes the absorption of light by leaves can be summarized, at least to the satisfaction of nonecologists, by the statement that ordinary green leaves absorb 75–90 per cent of the light in the red or blue part of the spectrum and that only very pale leaves absorb less than 50 per cent of the green light, where the absorption is least. Very dark leaves, such as those of *Ficus*, show 90–95 per cent absorption throughout the visible spectrum. Differences of this magnitude between various leaves seem to have little ecological significance. Since the rate of photosynthesis in nature may more often be dependent on carbon dioxide availability than on absorption of an adequate amount of light, it appears that the primary problem in the design of an efficient photosynthetic plant would probably be to provide for efficient carbon dioxide absorption from large volumes of air rather than for covering a large area with light-absorbing material.

Although the positions of the peaks of the absorption spectra of some pigments in leaves can be measured with fair precision, the same does not, however, apply to determinations of the true absorption as a function of wave length for the individual pigments in their native state. The difficulties are due to several causes. In the first place, there is a good deal of general absorption throughout the spectrum in leaves by what are often most unfortunately called "colorless components." This term would perhaps better be replaced by something more realistic, such as "residual brown matter." This probably includes well-known substances, such as flavones, absorbing primarily in the blue, as well as other colored materials. Another serious difficulty in the quantitative estimation of absorption spectra of individual pigments in leaves is the fact that the total pigment content is generally very high. This leads to

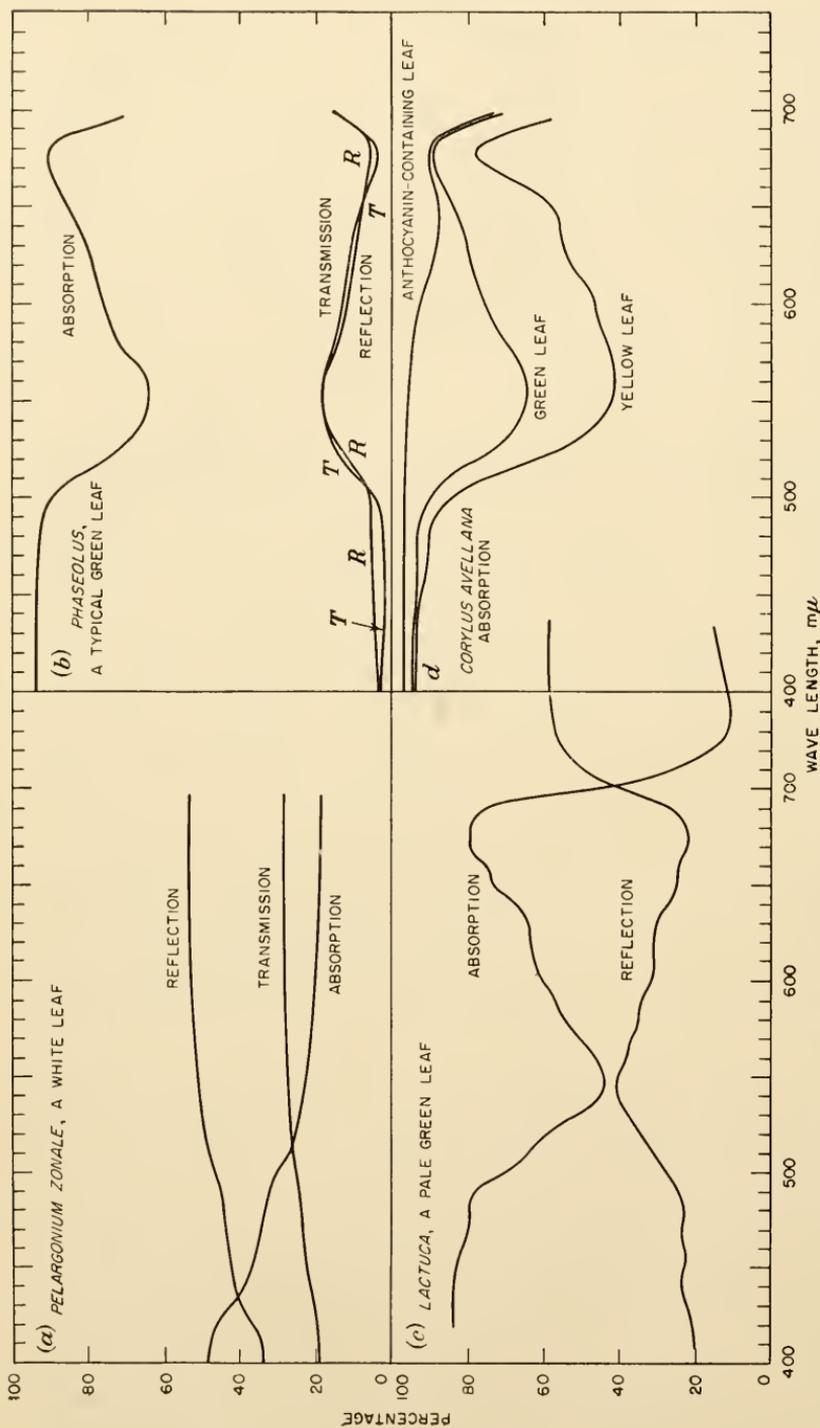


FIG. 6-2. The absorption, reflection, and transmission spectra of leaves. (a) A white leaf of *Pelargonium zonale*. (Seybold and Weissweiler, 1942a.) (b) A normal green bean leaf. (Seybold and Weissweiler, 1942a.) (c) A pale lettuce leaf. (Rabideau *et al.*, 1946.) (d) *Corylus avellana*: lower curve, yellow *Aureum* leaf (Seybold and Weissweiler, 1942a); middle curve, a darker green leaf; and top curve, a similar leaf with anthocyanin. The pigment contents, in milligrams per square centimeter, of these leaves were as shown in the table on page 351.

great inaccuracies in the measurement of absorption. An even greater difficulty is the overlapping of the absorption spectra of carotenoids and chlorophyll in the blue-green parts of the spectrum. For the purpose of evaluating the absorption due to the different pigments in leaves, these regions have thus far been nearly completely unusable. In fact, in this region it is often impossible even to recognize the appearance of distinct absorption peaks of pigments that are known to be present.

Let us first consider the absorption, reflection, and transmission spectra of a white leaf containing no chlorophyll. The striking thing about a "white" leaf, illustrated in Fig. 6-2a, is the high absorption, coming to nearly 50 per cent at the blue end of the spectrum and dropping to only about 20 per cent in the red. This may be taken as a very rough estimate of a base line to which in normal green leaves are added the absorption spectra of the chloroplast pigments. The reflection from this white leaf is about 50 per cent through most of the spectrum, a value that is reached only in the infrared by leaves that contain pigments. In normally pigmented leaves the reflection is greatly reduced and the absorption greatly increased in comparison with this white leaf. Since the total of the absorption, reflection, and transmission is equal to 1, the relation between these when the absorbing power is increased can easily be visualized. This particular leaf of Fig. 6-2a is somewhat unusual in being completely free of chlorophyll. Most "white" leaves actually contain a small amount of chlorophyll which can usually be seen clearly by observation of either absorption or fluorescence with a spectroscope, even though the green color is not obvious to the eye. In general, absorption throughout the spectrum, as illustrated by this white leaf, is largely responsible for the much higher absorption of leaves in the green part of the spectrum than would be predicted from the known absorption spectra of chlorophyll and the carotenoid pigments. The effect of this broad-band absorption throughout the spectrum upon photosynthesis measurements is discussed by Strain (1950). Measurements of a thin, light-colored lettuce leaf are presented in Fig. 6-2c. In the infrared the reflection and absorption of this leaf containing chlorophyll are much like those of the previously discussed white leaf, since the chloroplast pigments do not absorb in that region. This high reflecting power of leaves in the near infrared as compared with the high absorption in the visible part of the spectrum is the basis of the camouflage problems that depend upon matching the absorption and reflection spectra of paint with those of leaves. Ordinary green paint, although it may have absorption in the visible part of the spectrum rather similar to that of

	Chlorophyll a	Chlorophyll b	Carotene	Xanthophyll
Top curve.....	2.04	0.55	0.12	0.59
Middle curve.....	2.7	0.64	0.21	0.80
Lower curve.....	0.8	0.05	0.08	0.48

chlorophyll, does not reflect infrared to such a high degree as leaves do. Most leaves absorb far more light than this very pale lettuce leaf does. The bean leaf shown in Fig. 6-2*b* is much more typical of ordinary foliage. It will be noted that the reflection is quite low, amounting to less than 20 per cent even in the region around  $550\text{ m}\mu$ , where the absorption is the smallest. Five to ten per cent is more or less normal in red and blue, and the reflection rarely reaches a value so high as 20 per cent even in the green. In this particular leaf it happens that reflection and transmission are very nearly equal. In Fig. 6-2*d* we have a comparison in absorption among three leaves of *Corylus avellana*. The lower curve is for a leaf containing smaller amounts of pigment than the middle leaf. The top curve, similar otherwise to the middle leaf, contains a large amount of anthocyanin, a photosynthetically inactive red pigment.

In Fig. 6-3*a* is given the optical-density curve of the green alga *Chlamydomonas*, which is compared with the brown alga *Laminaria*. Both these curves have been adjusted to a density of 0.6 at the height of the red peak. The lack of chlorophyll b in *Laminaria* is clearly shown by the steepness of the red peak on the short-wave side and also by the shift in its position as compared with that in leaves and in *Chlamydomonas*. Furthermore the difference between the absorption of fucoxanthin in *Laminaria* and that of the carotenoids of *Chlamydomonas* is evident from the shoulder in the absorption curve of *Laminaria* at a wave length of about  $510\text{ m}\mu$ , whereas the principal carotenoid maximum in *Chlamydomonas* comes at about  $480\text{ m}\mu$ . A similar comparison may be made between the *Laminaria* curve and that for a leaf of *Aponogeton* in Fig. 6-3*b*. Also, in Fig. 6-3*b* is an absorption curve for an extract (presumably in methanol) of the same leaf which was prepared so as to contain the pigments in an area of solution equal to that of the leaf. These curves are therefore directly comparable. The same factor that was used in adjusting the leaf curve to a density of 0.6 was applied to the extract curve. Other comparisons by Seybold and Weissweiler (1942*a,b*) generally show a greater absorption in the live material than in the extract, even at the peaks. This set of data in which the peaks are nearly equal in height was selected so that the shapes of the two curves might be directly compared. It is obvious that a quantitative match of the leaf spectrum cannot be made by any simple modifications such as wave-length shifts and broadening of the absorption spectrum of the extract. A close approximation to the true optical-density curve of the pigment complex of leaves is, however, probably given by the curve in Fig. 6-3*c*. This represents the optical density of a water extract of ground spinach clarified by digitalin. This curve comes much closer to matching the absorption spectrum of living material than does that of the organic solvent extracts. This material contains both chlorophylls and carotenoids, presumably in combination with proteins. A chloro-

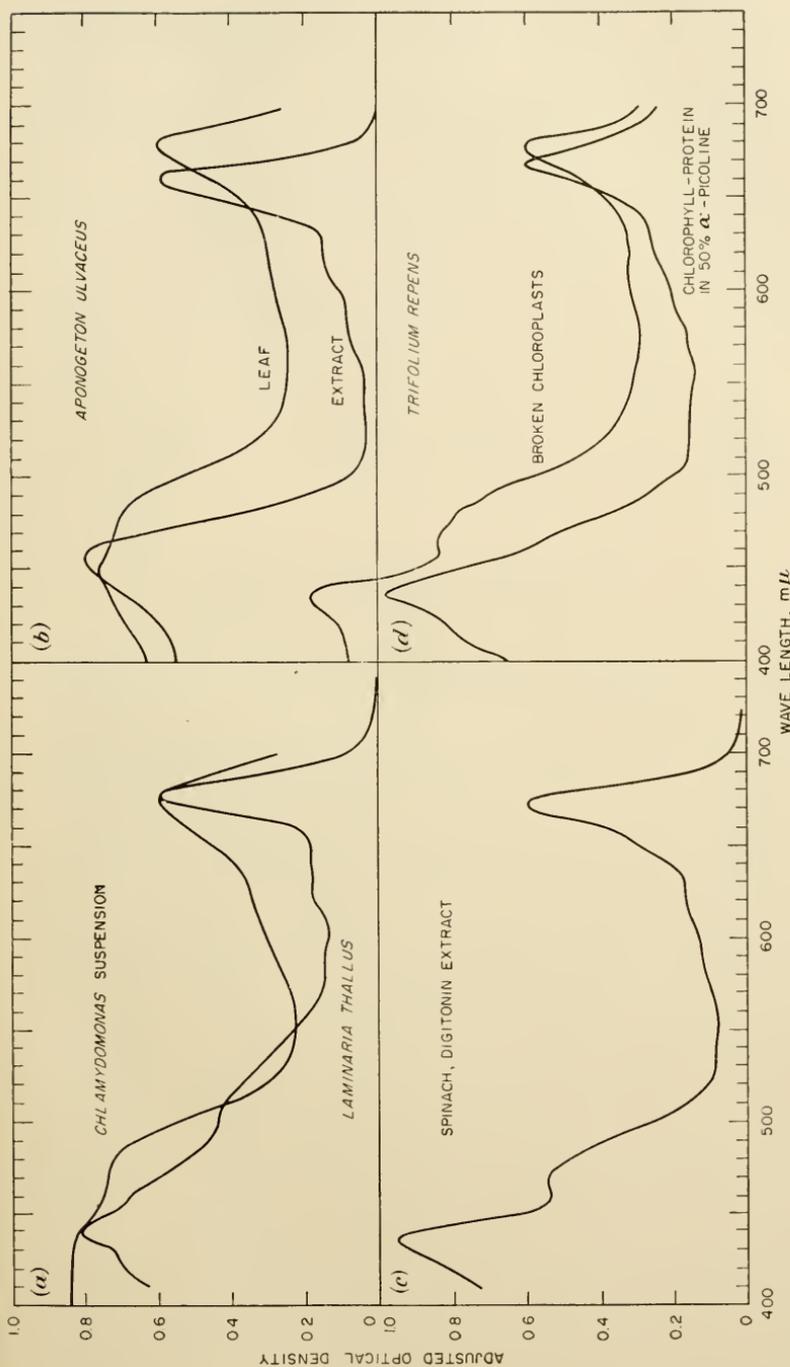


Fig. 6-3. Various absorption spectra, all adjusted to an optical density of 0.6 at the height of the red peak. (a) A green unicellular alga, *Chlamydomonas*, containing chlorophyll a and b (Seybold and Weissweiler, 1942a), and a brown alga containing predominantly chlorophyll a and fucoxanthin (Haxo and Blinks, 1950). (b) A comparison of a leaf of *Apogoneton ulvaceus* with an equal-area solution of the extracted pigments from the same leaf. The leaf-density curve was adjusted to 0.6 at the red peak, and the same factor used for the extract. (Seybold and Weissweiler, 1942a.) (c) A water extract of spinach ("phyllchlorin") solubilized with digitonin (Smith, 1938.) (d) Disintegrated chloroplasts of *Trifolium repens* suspended in water as compared with a carotenoid-free crystalline chlorophyll-protein preparation in 50 per cent  $\alpha$ -picoline. (Takashima, 1952.)

phyll protein has been obtained in crystalline form by Takashima (1952) in H. Tamiya's laboratory. This crystalline material, dissolved in 50 per cent  $\alpha$ -picoline, is shown in Fig. 6-3*d* in comparison with the suspension of broken chloroplasts from which it was prepared. The carotenoids have been removed, and, in addition, it is obvious from the shift in position of the red peak that some changes in the structure of the chlorophyll complex have taken place. The position of the peak at 435  $m\mu$  is, however, unaffected. What the relation may be between this crystalline derivative and chlorophyll in its native state in living organisms has not been determined. This is, however, the only chlorophyll-protein complex which has been isolated free of carotenoids and which has been crystallized. The crystals of this material remarkably resemble the appearance of sea urchins, being composed of a ball-like mass with numerous radial spikes. The molecular weight of this material was found to be about 19,200, the composition being about two molecules of chlorophyll per protein molecule.

#### ABSORPTION SPECTRA OF SOME PURIFIED CHLOROPLAST PIGMENTS

*Chlorophylls.* For quantitative determinations of chlorophyll a and b the measurement of the absorption in ether is preferable. The absorption curves of chlorophylls a and b in ether are presented in Chap. 7, Fig. 7-6. The absorption spectra of chlorophylls a and b in methanol are given in Fig. 6-4*a*. Although small amounts of water somewhat influence the height and shape of these curves, they are, nevertheless, very useful in the study of crude plant extracts in methanol. In addition, in Fig. 6-4*b* are given the curves for pheophytin a and b. Plant extracts, unless prepared with great care, may contain some chlorophyll that has been changed to pheophytin as a result of reaction with plant acids during extraction. Figure 6-4 also shows the absorption curves in methanol of chlorophyll c, found in dinoflagellates and brown algae, and of chlorophyll d, which is present in red algae. The participation of chlorophylls c and d in photosynthesis yet remains to be demonstrated.

*Carotenoids.* Descriptions of the individual carotenoids have been given by Strain (1949) with emphasis on the groups of plants in which the different carotenoids are found. The detailed chemistry of carotenoids is given by Karrer and Jucker (1950), and a monograph on the xanthophylls has been published by Strain (1938). The question of the function of carotenoids in various organisms, particularly in photosynthesizing plants, has received a great deal of attention, although at present the function of carotenoids in nonphotosynthetic organisms is not known. The relation of  $\alpha$ -carotene to vitamin A is the only obvious importance of the group of compounds aside from their connection with

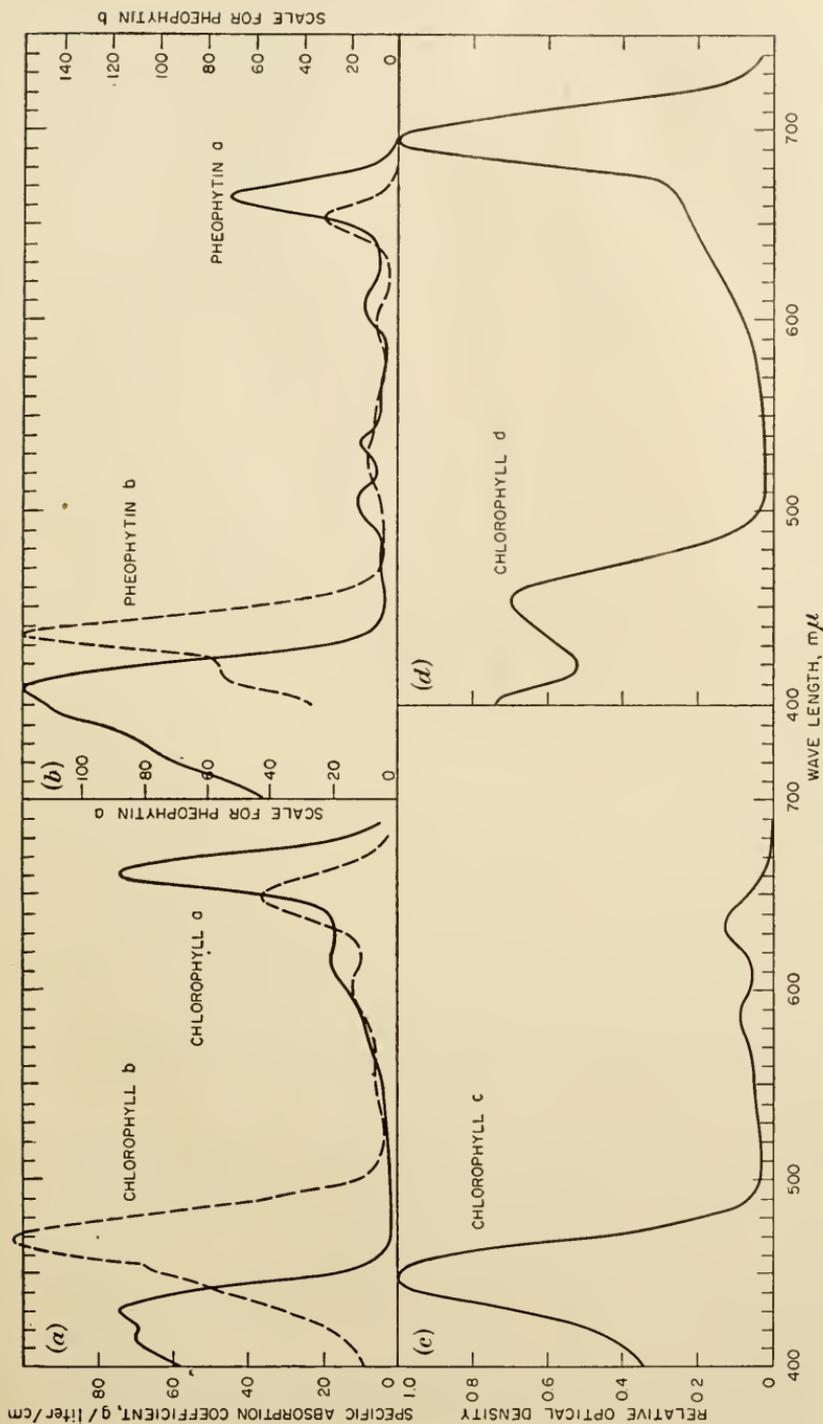


FIG. 6-4. The absorption spectra in methanol of various chlorophylls. (a) Chlorophylls a and b. (Harris and Zscheile, 1943.) The units for the absorption coefficient were selected so that the value at the red maximum would correspond to that given by Mackinney (1941). (b) Pheophytin a ( $M = 871.18$ ) and b ( $M = 885.16$ ) in methanol with 4 per cent ether. (Smith and Benitez, unpublished, 1954.) Specific absorption coefficient in square centimeters per gram. (c) Chlorophyll c, calculated to relative optical-density units from  $\log\text{-log } I_0/I$ . (Strain and Manning, 1942.) (d) Chlorophyll d, calculated to relative optical-density units from  $\log\text{-log } I_0/I$ . (Manning and Strain, 1943.)

photosynthesis. In some species it has been definitely demonstrated that certain carotenoids function in photosynthesis by the absorption of light, which is then transferred to chlorophyll. Absorption curves of a few of the more common carotenoids of particular significance in photosynthesis are presented in Fig. 6-5; the bacterial carotenoids will be discussed in a later section of this chapter.

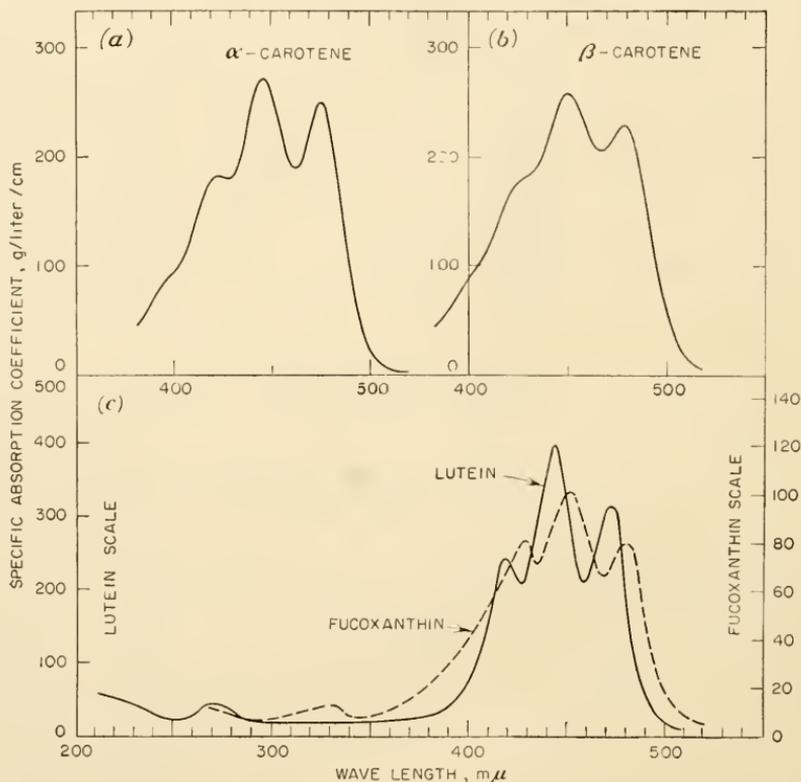


FIG. 6-5. The absorption spectra of a few common carotenoids in hexane. (a)  $\alpha$ -Carotene. (Zscheile *et al.*, 1942.) (b)  $\beta$ -Carotene. (Zscheile *et al.*, 1942.) (c) Lutein (xanthophyll) and fucoxanthin. (Karrer and Jucker, 1950.)

*Phycobilins.* The water-soluble phycobilins have not been investigated so thoroughly as the chlorophylls, since they are not so common, being restricted to red algae, blue-green algae, flagellates, dinoflagellates, and diatoms. Some of these pigments are extractable as water-soluble chromoproteins. Therefore the absorption curves of their extracts do not differ appreciably from those within the living organisms. These chromoproteins have the characteristics of plant globulins (Lemberg and Legge, 1949, p. 184). The prosthetic groups can be split off by drastic action of acid (Lemberg, 1929, 1930a) and have been identified as mesobiliviolin for phycocyanin and mesobilierithrin for phycoerythrin (*ibid.*;

Lemberg and Bader, 1933). Two separate phycoerythrins and two phycocyanins are usually recognized (Kylin, 1910; Lemberg and Legge, 1949, p. 146), although variations have been found in the reported positions of absorption maxima and their relative heights. R-phycoerythrin, the most common, has three absorption maxima in the visible range, at 566, 538, and 497  $m\mu$ , and C-phycoerythrin has a single maximum at 552  $m\mu$ . The designation of these two compounds as R- and C-phycoerythrin was suggested by Svedberg and Katsurai (1929), since R-phycoerythrin is more commonly found in Rhodophyceae and C-phycoerythrin in Cyanophyceae. The absorption curves of these pigments are shown in Fig. 6-6a and c. Higher specific absorption coefficients have been reported by Lemberg (1930a).

The absorption curves of two other phycoerythrins, presented by Svedberg and Eriksson (1932), are shown in Fig. 6-6a. The maxima in these curves appear to be in the same positions as those for R-phycoerythrin, but the relative heights of the three maxima are different. In curve 2 (phycoerythrin extracted from *Polysiphonia* and *Griffithsia*) the maximum at 497  $m\mu$  is nearly the same height as that at 565  $m\mu$ . In curve 3, for the phycoerythrin extracted from *Sebdenia*, only a shelf occurred at 538  $m\mu$ , and the 497- $m\mu$  maximum was much higher than that at 565  $m\mu$ . It is evident from these curves that the various phycoerythrins differ primarily in the relative proportions of the absorption that is contributed by the three separate bands. No definite difference in the wave length of the fluorescence of these modifications of phycoerythrin has been found (Svedberg and Eriksson, 1932), but the intensity of fluorescence of the *Polysiphonia* and *Griffithsia* phycoerythrin (curve 2) was much lower.

Absorption curves of C-phycoerythrin have been presented by Kylin (1931) and Boresch (1921). Boresch reports a single peak at 552  $m\mu$ . Kylin's curve shows a secondary maximum at about 496  $m\mu$ . The curve presented in Fig. 6-6c is the absorption of the autolysate of *Porphyridium cruentum* Naeg (V. K. Young, unpublished data). Whether the C-phycoerythrin absorption curve actually has shelves at 565 and 496  $m\mu$  or whether these shelves are due to the presence of small amounts of R-phycoerythrin in this organism is not known.

The two phycocyanins that are commonly recognized are R-phycocyanin, with two maxima at 614 and 551  $m\mu$ , and C-phycocyanin, with one maximum reported at 615  $m\mu$  by some investigators (Boresch, 1922; Svedberg and Katsurai, 1929; Lemberg, 1930a) and at 625  $m\mu$  by others. Lemberg and Legge (1949) suggest that these may be two separate pigments. Kylin (1912) has reported another phycocyanin with two maxima, at 610-615  $m\mu$  and 573-577  $m\mu$ , respectively, which he obtained from *Phormidium*. This is shown in Fig. 6-6b.

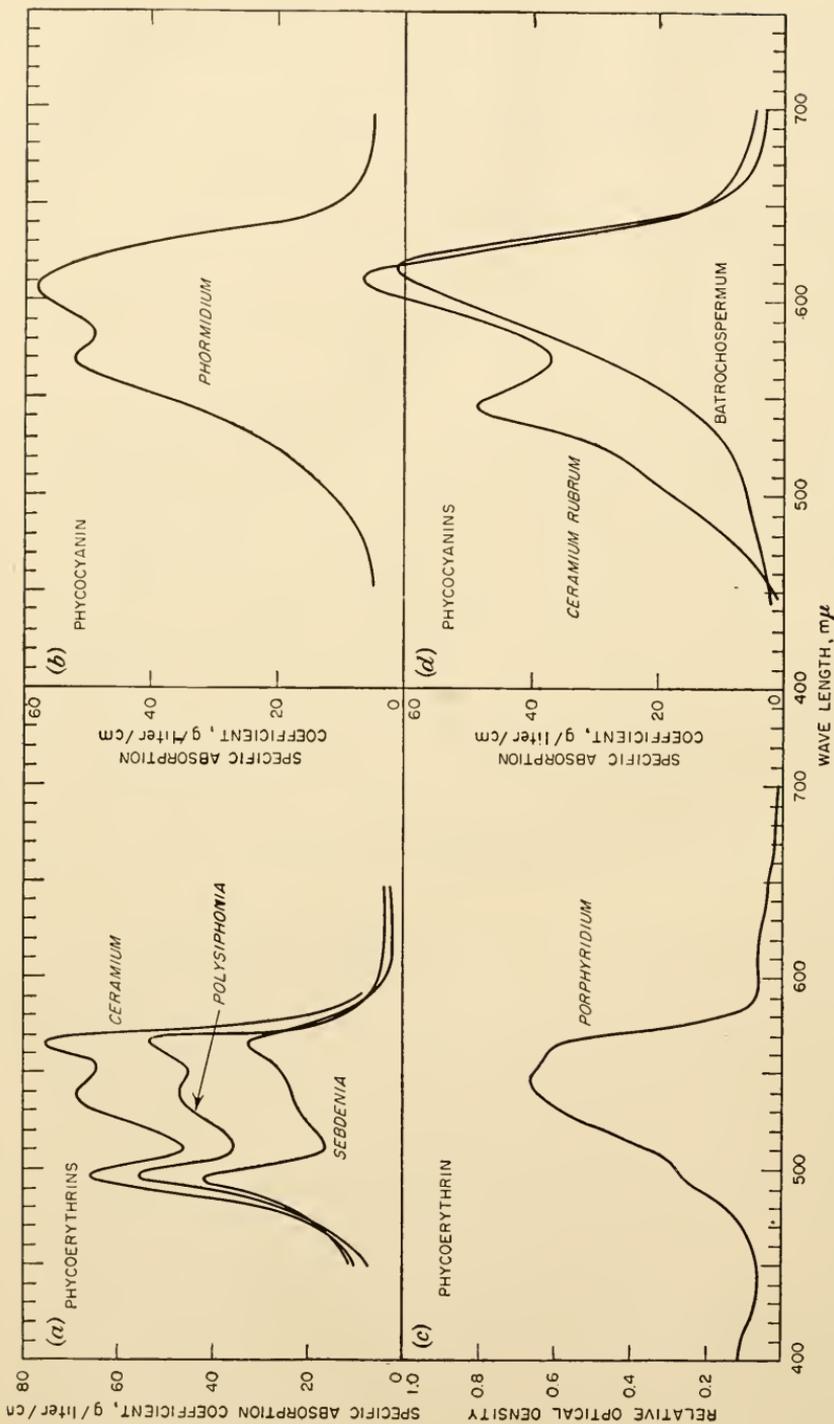


FIG. 6-6. Absorption spectra of phycobilins. (a) Specific-absorption curves of phycoerythrin from (1) *Ceramium*, (2) *Polysiphonia*, (3) *Sebdenia*. That from *Ceramium* is the phycoerythrin that has been called R-phycoerythrin. (Svedberg and Eriksson, 1932.) (b) Specific-absorption curve of blue phycocyanin extracted from *Phormidium*. (Kyllin, 1912.) (c) Relative-absorption curve of autolyse of *Porphyridium cruentum* Naeg. (V. K. Young, 1950 unpublished.) This organism has been reported to contain C-phycoerythrin. (d) Specific-absorption curves of (1) phycocyanin from *Ceramium rubrum* and (2) *Batrachospermum*. (Kyllin, 1911.)

## ACTION SPECTRA OF PHOTOSYNTHESIS

Seventy years ago the participation of a number of pigments as light absorbers in photosynthesis was clearly evident despite the fact that the methods available at that time did not give quantitative results and various sources of error were not taken into consideration. Englemann (1883, 1884), using motile bacteria for detection of oxygen evolution in red, blue-green, and yellow-brown algae, showed that light of spectral regions other than those absorbed chiefly by chlorophyll was active in photosynthesis. From these results he concluded that other pigments as well as chlorophyll were able to perform this function. Since then a number of workers have confirmed these results, e.g., Montfort (1934, 1936, 1941), Ehrke (1932), Schmidt (1937), and Levring (1947), to mention but a few. Since most of this type of work was done using broad spectral regions isolated by filters and since much more accurate data are now available, many papers of great significance for their time will not be discussed here. Photosynthesis action spectra have in recent years been obtained for green, red, brown, and blue-green algae using light of narrow band width. Emerson and Lewis used manometric methods for their determinations on *Chlorella* (1943) and *Chroococcus* (1942). Haxo and Blinks (1950) developed a polarigraphic method that permits the rapid determination of action spectra over wide ranges of wave lengths with biological variations kept at a minimum.

Figures 6-7 and 8 show absorption and action spectra of various organisms. In all these curves except those for red algae, chlorophyll a participation is evident from the maximum at 678  $m\mu$ .

It is a strange fact that, in spite of the number of papers on the effect of different wave lengths on photosynthesis, we have been unable to find a curve in the literature giving really reliable measurements of the rate of photosynthesis of a leaf of a higher plant at many different narrow wavelength bands. Figure 6-7a does, however, show corresponding action and absorption spectra for chloroplasts. The action measured by Chen (1951) was the reduction of an indophenol dye by a chloroplast suspension. This is one type of "Hill reaction" and is believed to be carried out by the same chloroplast components that are responsible for the photochemical step of photosynthesis. Part of the action spectrum, unfortunately one of the most interesting parts, is omitted here because the original data contain a point that appears to deviate from the curve. Chlorophyll b participation is evident from the 655- $m\mu$  hump. At 435  $m\mu$  the action peak is only 20 per cent lower than the absorption, which may be taken to mean that some of the carotenoids may be functional. Considerable inactive absorption is evident, however, from 400 to 430  $m\mu$ . The parts of the action curve which rise above the absorption curve may be attributed either to experimental error or to the presence of some

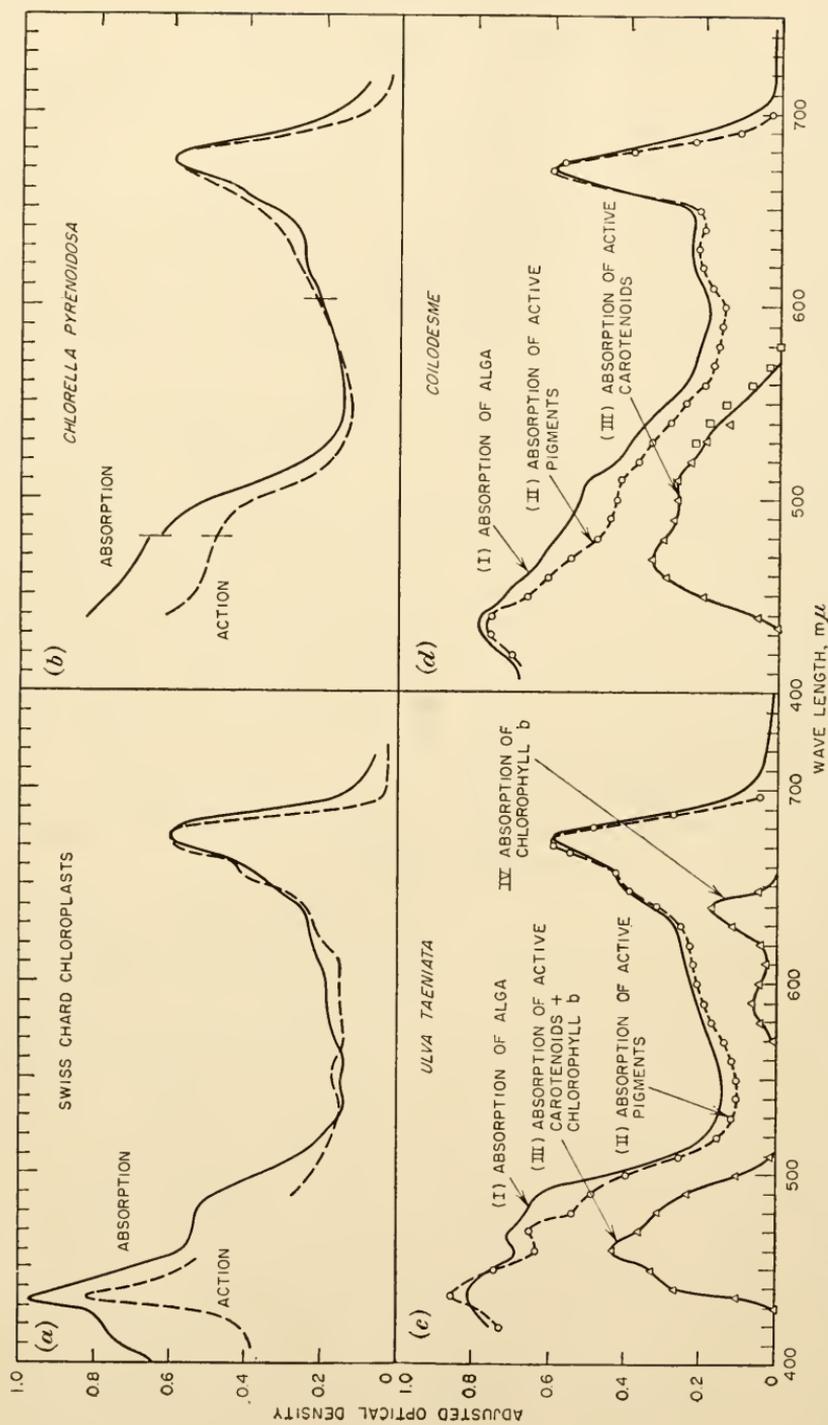


FIG. 6-7. Some action spectra of photosynthesis as compared with the absorption spectra of the same material, all converted to optical-density units and adjusted to a density of 0.60 at the red peak. (a) Action spectrum for dye reduction by a chloroplast suspension of swiss chard compared with its absorption. Part of the curve in the region of an aberrant point is omitted. (Data from Chen,

inactive absorption at the red peak, where the curves were matched in height.

The data of Fig. 6-7*b* of Emerson and Lewis for *Chlorella* photosynthesis and absorption were measured in three separate experiments that have been brought together here by factors to make complete curves. The participation of chlorophyll *b* is indicated by the shape of the action curve near 650  $m\mu$ , and that of some carotenoids by the shoulder at 480  $m\mu$ . Some inactive carotenoid absorption presumably causes the separation of the two curves below 500  $m\mu$ . Both in this figure and in that for spinach chloroplasts the action falls far below the absorption on the long-wave-length side of the chlorophyll red band. This widespread phenomenon, which has come to be known as the "Emerson effect," may be due to the energy of a quantum dropping below a minimum necessary value at about 690  $m\mu$ .

It is easy to demonstrate the participation of pigments that absorb in regions of the spectrum where others absorb very little or of pigments that are relatively efficient, such as the phycobilius. It is difficult, on the other hand, to detect the participation of pigments which are present in smaller quantity (i.e., chlorophyll *c*), which absorb in parts of the spectrum where other components absorb a greater share of the light, or which are relatively inefficient (e.g., chlorophyll *a* in Rhodophyceae). If inactive pigments are present in an appreciable concentration, they can distort the shape of the action curve, owing to their "internal-filtering" action, so that it is hard to interpret the action spectrum in terms of the active components. An example of this is seen in the action spectrum for protochlorophyll transformation to chlorophyll (Koski *et al.*, 1951), where the action spectrum for an albino lacking in carotenoids is compared with the action spectrum for normal seedlings.

Adequate corrections for internal filtering would in general greatly facilitate the interpretation of action spectra. Suitably corrected action

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1951.) (b) *Chlorella* photosynthesis and absorption. In this figure adjustments were made at the break points of the original data to bring the several sets together to a complete curve. (Data from Emerson and Lewis, 1943.) (c) *Ulva taeniata*. Curve I, thallus absorption converted to adjusted optical-density units. Curve II, optical density of the active components calculated from Eq. (6-9). Curve III represents an attempt to derive the absorption curve of the active carotenoids in *Ulva*. It was obtained by subtracting from curve II the calculated absorption of the active pigments of *Chroococcus* from Fig. 6-8*b*, which appears to be due, in the blue region, nearly exclusively to chlorophyll *a*. Curve IV is a similar approximation to the absorption spectrum of chlorophyll *b* in the 570- to 650- $m\mu$  region. It was obtained by subtracting the absorption of the active pigments of *Coilodesme*, which lacks chlorophyll *b*, from curve II. (d) *Coilodesme*, a brown alga. Curve I, thallus absorption converted to adjusted optical-density units. Curve II, absorption curve for the active components calculated from Eq. (6-9). Curve III, absorption curve of active carotenoids, which was obtained by subtracting the curve of *Chroococcus* from the blue end of the *Coilodesme* absorption spectrum. (Haxo and Blinks, 1950.)

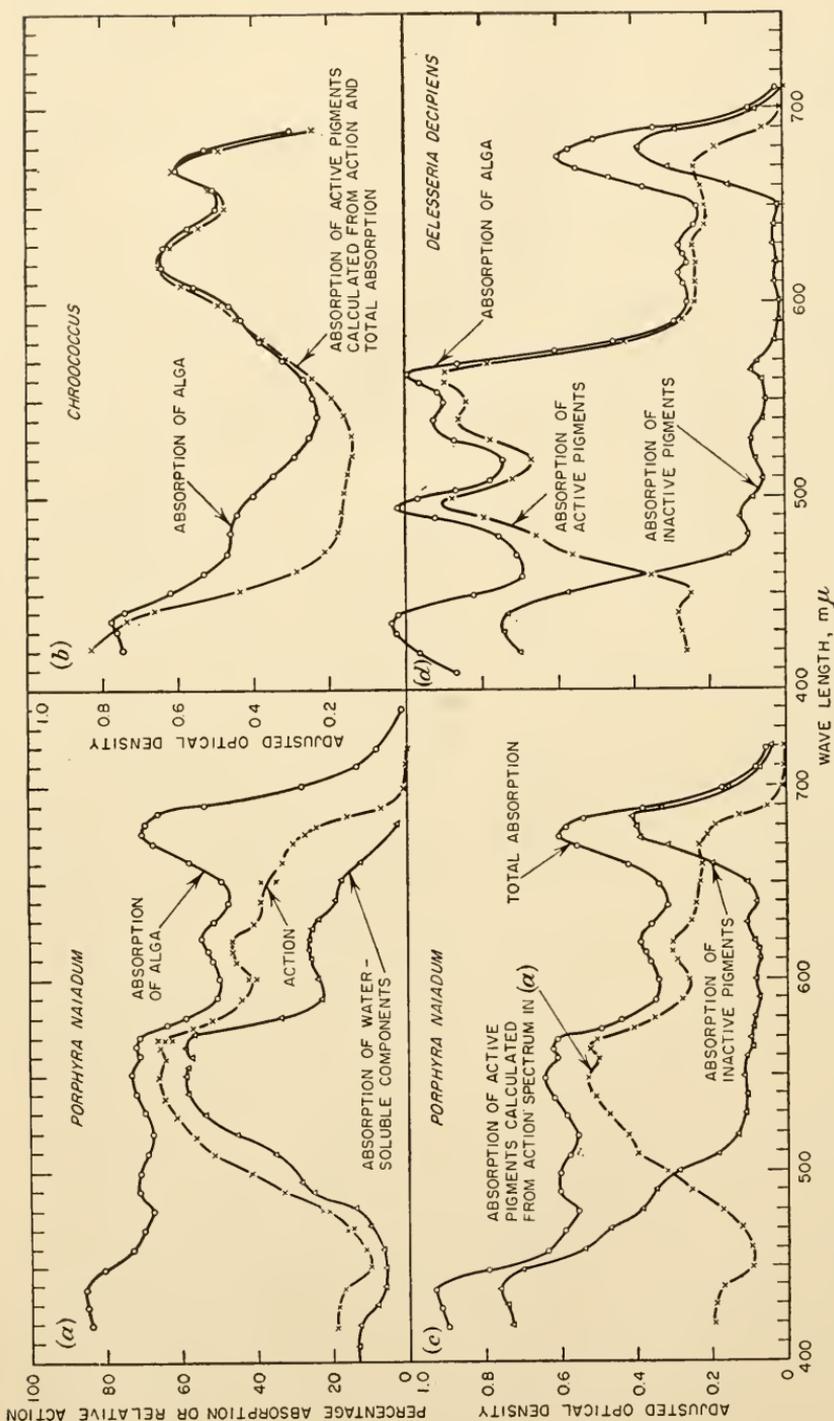


FIG. 6-8. (a) *Porphyra naiadum*. Photosynthetic action and percentage absorption curves. (Original data of Haxo and Blinks, 1950, replotted.) (b) Optical density of the algal suspension and calculated optical density of active pigments in *Chroococcus*. (Data from Emerson and Lewis, 1942.) (c) Optical-density curves for total pigments, active pigments, and inactive pigments in *Porphyra naiadum*, calculated from the absorption and action spectra in part a. (d) Optical-density curves for total pigments, active pigments, and inactive pigments in *Delesseria decipiens*, calculated from data of Haxo and Blinks (1950).

spectra could be used to compute the absorption curves of active pigments *in vivo*. Few such curves are available at the present time, but by suitable selection of organisms this method could yield badly needed data. Several investigators (Dutton and Manning, 1941; Emerson and Lewis, 1943) have used extracted pigments for the determination of the partition of light among various components. This is not entirely satisfactory, however, since shifts of position and of the shape of the absorption curves are known to occur in the living organism as compared with extracts.

An equation<sup>2</sup> can be derived for calculating the optical densities of the active pigments from the absorption and action spectra. Data so treated give absorption spectra of the active pigments corrected for the distortion introduced by the inactive pigments.

As an illustration, the equation has been used on the *Coilodesme* and *Ulva* curves (see Fig. 6-7). The absorption curve for chlorophyll a *in vivo* is not known in the blue part of the spectrum; however, a close

<sup>2</sup> Derivation of the equation for calculating the optical density of the active pigment from an action spectrum and an absorption spectrum: Let

$F$  = fraction of incident monochromatic light absorbed by whole system.

$F_a$  = fraction of  $F$  absorbed by active pigments.

$K$  = proportionality constant which cancels out.

$D$  = optical density of whole system.

$D_a$  = optical density of active pigments.

$P$  = rate of photosynthesis or other action under such conditions that it is proportional to quantum intensity of light absorbed by active pigment.

$I$  = incident light intensity.

Primes designate the quantities at a particular wave length where all absorption is due to the active pigment, as, for instance, at the red absorption maximum of chlorophyll. The fraction of absorbed light absorbed by the active pigment is given (Kistiakowsky, 1928, p. 39) by

$$F_a = D_a/D, \quad (6-1)$$

$$P' = KIF', \quad (6-2)$$

$$P = KIFF_a. \quad (6-3)$$

The ratio of Eq. (6-2) to (6-3) is

$$P'/P = KIF'/KIFF_a = F'/FF_a, \quad (6-4)$$

or

$$F_a = F'P/P'F. \quad (6-5)$$

Substitution of Eq. (6-1) in Eq. (6-5) gives

$$D_a/D = F'P/P'F, \quad (6-6)$$

or

$$D_a = F'PD/P'F. \quad (6-7)$$

Since, by Beer's law,

$$D = \log [1/(1 - F)], \quad (6-8)$$

$$D_a = F'P/P'F \log [1/(1 - F)]. \quad (6-9)$$

approach to it, in the region 420–520  $m\mu$ , is the optical-density curve for the active component in *Chroococcus* (Fig. 6-8b). Subtracting this curve from that of the active components in *Coilodesme* (Fig. 6-7dII), we obtain data that should represent the optical density of the active carotenoids (see Fig. 6-7dIII). It is realized that this curve can be improved upon with more detailed measurements, which would give a better chlorophyll a curve.

The active-absorption curve obtained for chlorophyll a between 580 and 700  $m\mu$ , calculated from *Coilodesme* that contains no chlorophyll b, has been used similarly to subtract from the curve for the active components in *Uva*. The result should represent an approximation to the absorption curve of chlorophyll b in living algae (see Fig. 6-7c).

In the action spectrum of Haxo and Blinks (1950) for photosynthesis of *Porphyra naiadum* (see Fig. 6-8a), the photosynthetic function of phycoerythrin (500–570  $m\mu$ ) and of phycocyanin (620  $m\mu$ ) is seen to exceed that of chlorophyll (430 and 670  $m\mu$ ). The phycoerythrin of *Porphyra naiadum* is different from other phycoerythrins in that its 490- $m\mu$  band is inconspicuous. In Fig. 6-8c is the absorption spectrum of the active and, by difference, that of the inactive pigments in this species, as calculated from Eq. (6-9). Strangely enough, the curve for the inactive pigments is composed mainly of chlorophyll but does also include some carotenoid absorption around 500  $m\mu$ . In some unpublished experiments C. Yocum and L. R. Blinks (1950) have found that red algae may be adapted by growth in red light so that the inactive chlorophyll becomes functional. This adaptation process is reversed if the algae are exposed to strong green light. An illustration of photosynthesis due to absorption of light by phycocyanin as well as by chlorophyll and to inactive absorption by a carotenoid is found in Fig. 6-8b, calculated from the data of Emerson and Lewis (1942).

A red alga with a high content of phycoerythrin and a low phycocyanin content, *Delesseria decipiens*, was one of the many species used in the work of Haxo and Blinks. Curves for the active and inactive pigments of this alga have been calculated from their data and are given in Fig. 6-8d. The derived absorption curve for the inactive pigments of this alga seems to be a reasonable approximation to that curve so basic to the present subject—the *in vivo* absorption spectrum of chlorophyll a.

## FLUORESCENCE SPECTRA OF PHOTOSYNTHETIC PIGMENTS

### FLUORESCENCE SPECTRA OF THE EXTRACTED PIGMENTS

Absorption spectroscopy is widely used for the identification and quantitative determination of biological pigments. Far less use has been made of fluorescence spectroscopy, largely because of the lack of adequate com-

mercial equipment for precise measurement of the energy distribution of the very weak light produced by fluorescent pigments. In general terms it may be said that fluorescence spectroscopy is just as useful for the qualitative identification of pigments as absorption spectroscopy is, assuming, of course, that the pigments of interest do fluoresce. It is probably not so useful as absorption spectroscopy for quantitative measurements of pigment concentration. There is, however, an important type of information that can be obtained only from fluorescence spectra, and that is the distribution of energy between various pigments in a complex mixture of fluorescent substances which is being irradiated. Such applications will be taken up in the following section after the fluorescence spectra of the photosynthetic pigments have been discussed.

Chlorophyll in solutions of organic solvents gives a brilliant red fluorescence whose spectral energy distribution has been measured by Zscheile and Harris (1943). Two of their curves for chlorophyll a fluorescence spectra in ether are given in Fig. 6-9a, which also shows the absorption spectrum of chlorophyll a in the same solvent. The tall fluorescence curve obtained in a dilute solution shows the true spectrum in ether. The great distortion of the fluorescence curve which can be introduced by reabsorption of the fluorescent light by the pigment itself is observed in the small curve obtained through a thick layer of solution. The reabsorption changes the apparent position of the peak that lies close to the absorption band and also greatly reduces its height in comparison with that of the 725-m $\mu$  band, which is hardly affected. A similar effect of reabsorption is found in a comparison of the spectra of chlorophyll in a pale and in a dark green leaf in Fig. 6-10c. Figure 6-9b gives the absorption and fluorescence spectra of chlorophyll b in ether. Watson and Livingston (1948) discuss the self-quenching of chlorophyll fluorescence.

Although different absorption spectra are found for phycoerythrin from various species, the fluorescence spectra are identical for at least two samples having very different absorption spectra. Phycoerythrin from *Porphyridium cruentum* has the spectra given in Fig. 6-9c, and that from *Porphyra* kindly given us by Prof. L. R. Blinks shows a different absorption spectrum but a nearly identical fluorescence spectrum. In Fig. 6-9c the crosses represent the effectiveness of different wave lengths in exciting the fluorescence of this solution of phycoerythrin (V. K. Young, 1950, unpublished data). The absorption and fluorescence spectra of phycocyanin from *Oscillatoria* (Duysens, 1951a) are shown in Fig. 6-9d. The fluorescence spectra of the photosynthetic bacteria will be given in the last section of this chapter. There have been some reports of carotenoid fluorescence, but if it does exist, it is not readily observable in the common representatives of that class of compounds either in live cells or in extracts.

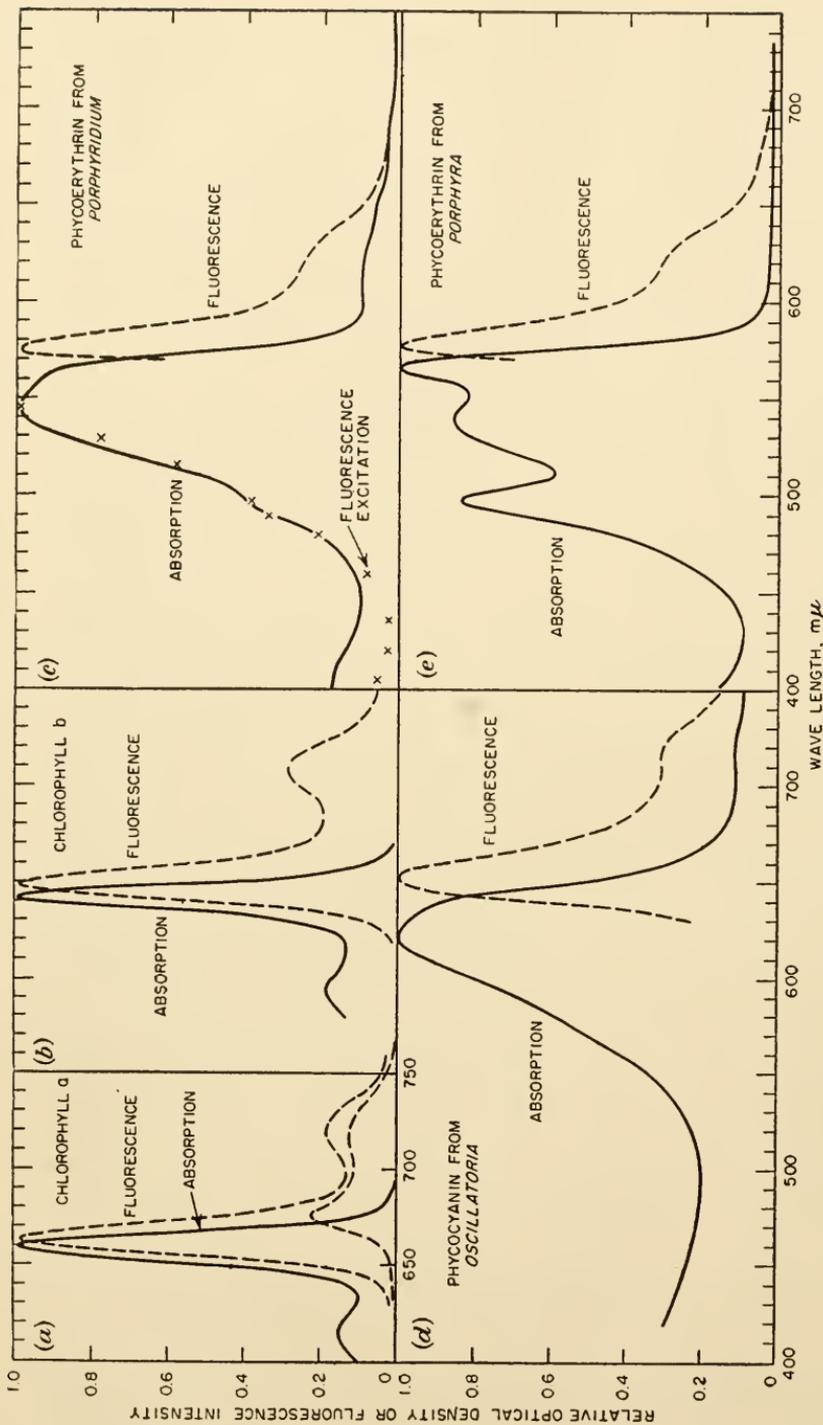


FIG. 6-9. The fluorescence and absorption spectra of extracted photosynthetic pigments. (a) Chlorophyll a in ether. The influence of reabsorption on the shape of the fluorescence curve is shown in the small curve [absorption (Zscheile and Comar, 1941), fluorescence (Zscheile and Harris, 1943)]. (b) Chlorophyll b in ether [absorption (Zscheile and Comar, 1941), fluorescence (Zscheile and Harris, 1943)]. (c) Phycoerythrin extracted from *Porphyridium cruentum*. The crosses indicate the effectiveness spectrum for fluorescence excitation of this solution. (V. K. Young, unpublished.) (d) Phycoerythrin from *Oscillatoria*. (Duggens, 1951a.) (e) C-phycoerythrin prepared by L. R. Blinks (unpublished).

TRANSFER OF ENERGY BETWEEN PIGMENTS IN LIVE PLANTS  
AS DETERMINED BY FLUORESCENCE SPECTROSCOPY

The manner of the participation of various pigments in photosynthesis has long been a question of interest. Since all photosynthetic organisms with the exception of bacteria contain chlorophyll a, it has been proposed that the other pigments might function by transferring their absorbed energy to chlorophyll. One way to test for the occurrence of this energy-transfer process is to see if light absorbed by the other pigments can cause chlorophyll to fluoresce in the live cells. This method was used by Wassink and Kersten (1944) and by Dutton *et al.* (1943) with the diatom

TABLE 6-1. THE EXCITATION OF CHLOROPHYLL FLUORESCENCE BY LIGHT  
ABSORBED BY CAROTENOIDS IN *Nitzschia* AND IN *Chlorella*

Organism	Incident wave length, A	Absorbed light absorbed by chlorophyll, %	Calculated fluorescence-yield ratio		Observed ratio
			No energy transfer	Complete transfer	
<i>Nitzschia closterium</i> .....	4700 vs. 5780 or 6000	26 vs. 95 or 96	0.27	1.0	1.2 ± 0.2
	4358 vs. 5780 or 6000	51 vs. 95 or 99	0.53	1.0	1.1 ± 0.2
<i>Chlorella pyrenoidosa</i> .....	4700 vs. 5780 or 6000	52 vs. 100	0.52	1.0	1.05 ± 0.04
	4358 vs. 5780 or 6000	81 vs. 100	0.81	1.0	0.93 ± 0.18

*Nitzschia*, which is rich in yellow pigments, especially fucoxanthin, and with *Chlorella pyrenoidosa*. They calculated the fluorescence-yield ratios at wave lengths absorbed by chlorophyll alone and at some wave lengths absorbed by both chlorophyll and carotenoids. The results obtained by Dutton and Manning are given in Table 6-1. They indicate energy transfer from carotenoids to chlorophyll even though some error tending to increase the apparent amount of energy transfer may have been introduced as a result of reabsorption of fluorescent light within the cells.

An attempt was made to measure energy transfer in red algae in a similar manner (Van Norman *et al.*, 1948) to determine whether phycoerythrin transferred energy to chlorophyll or took part directly in the photosynthetic mechanism without the intermediate assistance of chlorophyll. These algae presented a more complicated situation, however, since the phycoerythrin and phycocyanin also fluoresce and their fluorescence overlaps that of chlorophyll, so that it is difficult to determine how much of the red fluorescence is due to chlorophyll.

Duysens (1951a,b, 1952) has made measurements of the energy transfer from phycoerythrin to phycocyanin and to chlorophyll as well as from phycocyanin to chlorophyll in red algae. The efficiency for the first transfer appeared to be probably greater than 80 per cent; the efficiencies for the transfer from the phycobilins to chlorophyll were found to be equal.

In the unicellular red alga *Porphyridium cruentum* the fluorescence spectra have been measured for various incident wave lengths. Figure 6-10 shows a family of fluorescence curves obtained by using equal quanta of incident light of various wave lengths to excite fluorescence in a suspension of *Porphyridium cruentum* Naeg. The sizes of the curves have been adjusted so that the curves can be compared directly on the basis of equal numbers of incident quanta of the different wave lengths. These and other comparable curves were resolved into the fluorescence spectra of the individual *in vivo* pigments, as illustrated in Fig. 6-10d. The chlorophyll fluorescence excited by 546  $m\mu$  is 3.8 times as intense as that excited by an equal number of quanta at 436  $m\mu$ . The effectiveness curves derived from the above data for the excitation of chlorophyll, phycoerythrin, and phycocyanin fluorescence in the live cells are shown in Fig. 6-10a (French and Young, 1952). The action spectra show that in algae illuminated with blue light there is some chlorophyll fluorescence due to light absorbed directly by chlorophyll, as evidenced by the small peak around 440  $m\mu$  in the chlorophyll excitation spectrum. We attribute the small size of this peak to internal filtering by carotenoid pigments.<sup>3</sup> The rise in these three curves from 450 to 500  $m\mu$  matches the absorption band of phycoerythrin in these algae (Fig. 6-9c). This is taken to mean that energy absorbed by phycoerythrin can be transferred to chlorophyll and that phycocyanin may be an intermediate in this transfer process.

Energy transfer has also been demonstrated by Duysens (1951a,b, 1952) from carotenoids to bacteriochlorophyll in *Chromatium*. Figure 6-15c shows the action spectrum for fluorescence and phototaxis in this organism.

In resolving the fluorescence excitation spectra into their individual components, it is necessary to know whether the fluorescence yield of each separate pigment is independent of the wave length of the incident light. Organisms containing only one pigment would be valuable in this type of analysis. Such organisms have not become available yet (the one reported case being the  $w_3$  mutant of *Zea mays* L., which contains other pigments than protochlorophyll in only very minute traces). Infor-

<sup>3</sup> Duysens (1951b) finds, however, that, even after a correction for internal filtering by carotenoids is applied, the fluorescence of chlorophyll excited by blue light directly absorbed by chlorophyll is smaller than the fluorescence excited by transfer from the phycobilin.

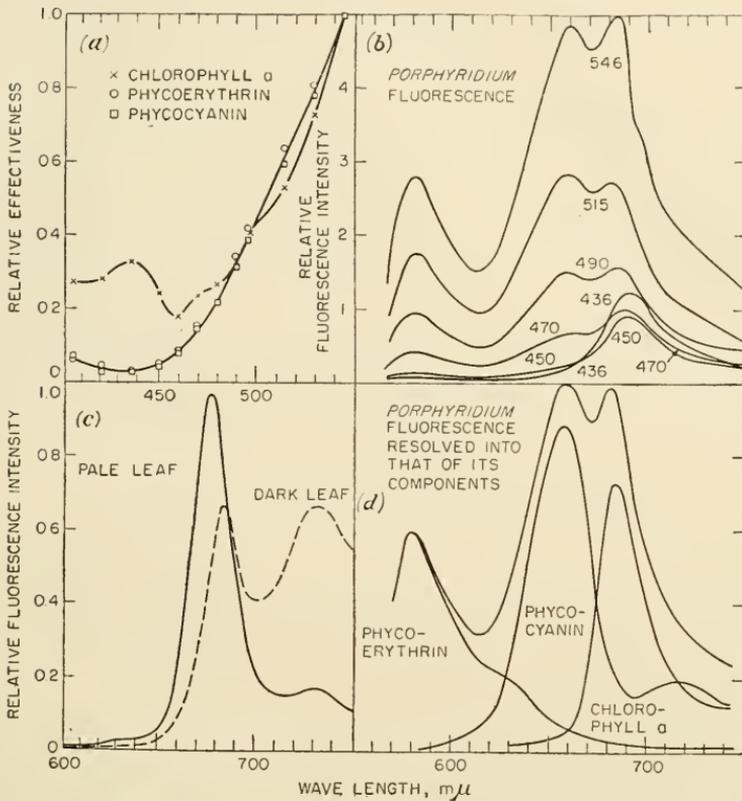


FIG. 6-10. (a) Effectiveness spectra for the excitation of fluorescence of chlorophyll a, phycoerythrin, and phycocyanin in *Porphyridium cruentum* Naeg. These curves were adjusted to the same height at wave length 546 mμ. (b) Fluorescence spectra of *Porphyridium cruentum* illuminated by different wave lengths. The curves are adjusted to show their relative size for equal numbers of incident quanta. These and other curves of the same family, when analyzed as in part d, gave the data from which the points of part a were plotted. (c) The fluorescence spectra of a very pale green corn leaf and a darker green leaf of *Dendromecon rigida* excited by a wave length of 436 mμ. The corn leaf shows the true fluorescence spectrum of chlorophyll a *in vivo*, and the other shows the distortion due to reabsorption of the fluorescence, indicated by a lowering of the main peak accompanied by a shift of its position toward the red end. The height of the 730-mμ peak, which is relatively less influenced by reabsorption, is greater, the higher the chlorophyll concentration. (d) The fluorescence spectrum of *Porphyridium* excited by a wave length of 530 mμ resolved into the spectra of the three separate fluorescing pigments. The three lower curves, which add to give the curve for the intact algae, represent the fluorescence spectra of phycoerythrin, phycocyanin, and chlorophyll a in these live algae. (French and Young, 1952.)

mation relevant to this problem must therefore be obtained from solutions of individual components. The data in this regard are rather nebulous. Prins (1934) reported a lower yield for chlorophyll fluorescence in the blue region of the spectrum than in the red. Watson and Livingston (personal communication, 1950) find that the yield of chloro-

phyll a fluorescence with exciting light of 4358 Å is approximately 0.6 and, with 6685 Å, 0.8 of the yield with 5780 Å. A lower yield at the longer wave lengths (i.e., 690  $m\mu$ ) has been known for some time and has been demonstrated for other compounds as well as chlorophyll (Pringsheim, 1949). This whole subject needs further investigation.

The transfer of energy in solutions of these pigments is another field that has not been sufficiently explored. Watson and Livingston (1950) have reported energy transfer from chlorophyll b to chlorophyll a in equimolar solutions at molarities greater than  $2 \times 10^{-4}$ . The molar concentration of chlorophyll in living leaves of some species has been calculated to be 0.2 (Aronoff, 1950), so that the transfer of energy is certainly theoretically possible at this concentration. Duysens (1951b) also found the transfer of energy from chlorophyll b to chlorophyll a in solution.

### SPECTRA OF PHOTOSYNTHETIC BACTERIA

Photosynthetic bacteria are organisms that are of relatively little quantitative importance in the carbon cycle of nature. These bacteria are, however, of great interest as experimental material for the investigation of photosynthesis. They are fairly widespread, being found in mud and in ditch water, but rarely occur in large quantity. Since these organisms have a photosynthetic mechanism that differs in some major respects from that of higher plants and since their pigments are different from those in higher plants, they have provided excellent material for the application of the principles of comparative biochemistry to the study of photosynthesis. Their study has therefore contributed to a more generalized understanding of the photosynthetic process (Van Niel, 1941, 1944). The photosynthesis of these bacteria differs from that of higher plants in that they use other compounds than water for reductants, namely, hydrogen sulfide, thiosulfate, hydrogen, or other inorganic or organic compounds. The pigments present are analogous to, but not identical with, those in leaves of green plants. The chlorophyll component in the purple bacteria is called "bacteriochlorophyll" and in green bacteria, "bacterioviridin." The carotenoids also differ in chemical structure from that of higher plants. For the following reasons the spectral absorption characteristics of these bacteria have made them interesting material for the study of the function of photosynthetic pigments: (1) since bacteriochlorophyll absorbs wave lengths longer than those absorbed by chlorophyll a, extension of the study of absorption, fluorescence, and action spectra into the near infrared has been possible; (2) bacteriochlorophyll exhibits different absorption properties in various strains of purple bacteria, thus providing a means for the study of the physical state of this photosynthetic pigment in living organisms; (3) the absorption maxima of the different pigments are well separated in bac-

teria as compared with leaves and algae, thus simplifying the interpretation of both absorption and action spectra.

#### ABSORPTION SPECTRA OF PHOTOSYNTHETIC BACTERIA AND OF THEIR PIGMENTS

Before taking up the purple bacteria, let us first consider an intermediate form, the green bacteria. These contain a pigment somewhat similar to chlorophyll a but not identical with it. The absorption spectra of the acetone extract and of a suspension of green bacteria are shown in Fig. 6-11. It is obvious from this figure that the carotenoid content of this organism is very small. A comparison of the absorption spectrum of the acetone extract with that of the suspension of the bacteria themselves shows a very large shift in the wave length of the red peak of bacterio-*viridin* when it is extracted from the living bacteria. The position of this peak, about 740  $m\mu$  in the living bacteria, falls between the red absorption maximum, 680  $m\mu$ , of green leaves, and the 800- to 900- $m\mu$  region in which the purple bacteria absorb energy and carry on photosynthesis. A detailed study of the quantum yield of photosynthesis in the green bacteria has been made by Larsen *et al.* (1952), to whom we are indebted for Fig. 6-11.

The absorption spectra of many species of purple bacteria in the near infrared have been examined by Katz and Wassink (1939), Wassink *et al.* (1939), Wassink and Manten (1942), and Giesberger (1947). Most of these measurements were made by pressing the cells in thin layers between glass plates. The lack of an appropriate scattering correction in these spectra introduces appreciable distortion in shape, but nevertheless the band position is very well defined, and even the relative peak heights in adjacent wave-length regions were obtained by this means. Bacteriochlorophyll bands were found in several different regions of the spectrum. Bands at 890-875  $m\mu$ , at 860-840  $m\mu$ , and at 803-795  $m\mu$  have different relative intensities in different species. All the species investigated, however, had an absorption band at 590  $m\mu$  which was due to bacteriochlorophyll. According to Duysens (personal communication, 1952), an absorption band of bacteriochlorophyll occurs in the near-ultraviolet region. This region, however, is not represented in the spectra of the afore-mentioned authors. A most striking fact was found by Wassink and his collaborators (1939) when bacteriochlorophyll was extracted from these different species of purple bacteria by organic solvents. Whatever the position of the absorption bands in the bacteria, the bacteriochlorophyll extracted from all these species was spectroscopically identical. It is presumed that the position of the bands in the living cells is due to combination of the bacteriochlorophyll with some other cell substance, probably a protein, and that several different proteins or different modes

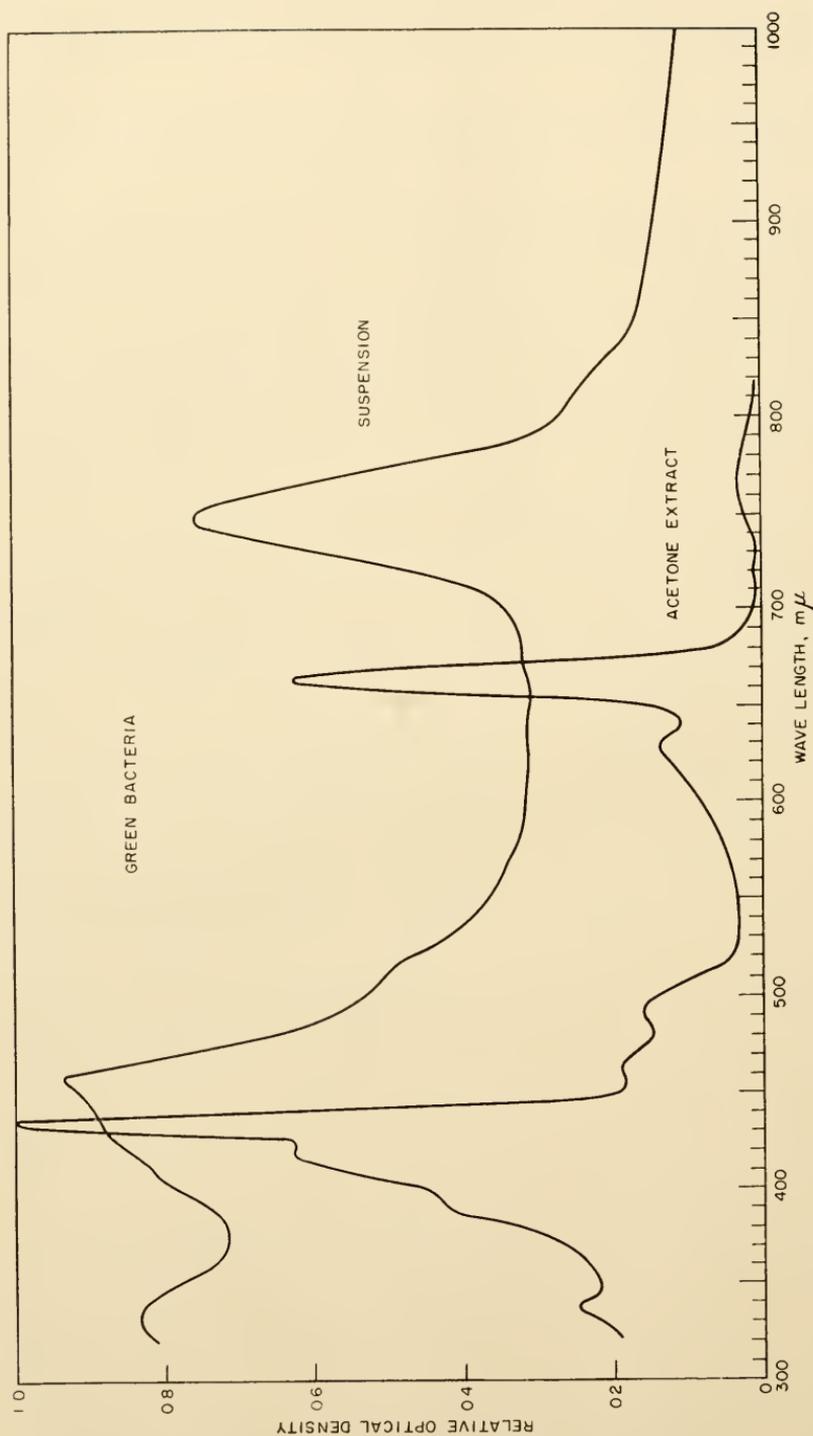


Fig. 6-11. The absorption spectrum of a suspension of photosynthetic green sulfur bacteria as compared with that for an acetone extract of the same material. (Larsen, personal communication, 1951.)

of combination are present even in a single species of these bacteria, thus causing the absorption peaks to occur in various positions.

Katz and Wassink (1939) have also investigated aqueous extracts of photosynthetic bacteria containing the pigments in a water-soluble form combined, presumably, with protein. These aqueous extracts, prepared either by grinding or by the use of supersonic vibration, can be clarified by the addition of urea and probably also other detergents without shift of the band position from that of the live cells. The absorption spectra of several such preparations from different species are shown in Fig. 6-12 (French, 1940a). These measurements were made in a spectrophotometer in which the photocell was placed very close to the solution; this further reduced the likelihood of distortion by light scattering. These curves also show large variations in relative height of the 790- and the 850- $m\mu$  absorption bands and perhaps small variation in position. In the *Phacomonas* curve the infrared band shows a pronounced shoulder at about 870  $m\mu$ . More data for the absorption spectra of the bacterial extract and of the intact bacteria are shown in Figs. 6-13 and 15. The infrared part of the absorption spectrum of these bacteria on a frequency plot has been analyzed as sums of three to five symmetrical components by Wassink *et al.* (1939). Variations in the relative proportions of these different bands lead to the wide variation in the shape of the bacterial-absorption curves. Between 420 and 550  $m\mu$  a large part of the absorption of these bacteria is due to the presence of carotenoid pigments. Comparisons of the absorption in this region in Figs. 6-12 through 15 show that the nature of the carotenoids in different species varies greatly.

In Fig. 6-14 the absorption curves of some of the isolated purple bacteria carotenoids in benzene solution are shown. The best known of these is spirilloxanthin, studied by Van Niel and Smith (1935) and by Polgár *et al.* (1944), which occurs in *Rhodospirillum rubrum*. Parts *c*, *d*, and *e* of Fig. 6-14 show the other carotenoids isolated chromatographically by Manten (1948) from *Rhodospirillum rubrum*. These pigments are present in far smaller quantities than spirilloxanthin is, but it is evident from the action spectra that one or more of these pigments rather than the spirilloxanthin itself are responsible for what activity the carotenoids may have in photosynthesis.

A particularly interesting situation occurs in *Rhodopseudomonas spheroides*, formerly known as *Streptococcus varians*. This species when grown anaerobically is brown. When grown aerobically, it is bright red. An anaerobic culture may be turned red by shaking with air. The red pigment once formed is not turned back to brown by anaerobic incubation. The nature of this pigment change has been investigated in the live bacteria by French (1940b) and by Van Niel (1947), who isolated the pigments. The absorption spectra of the two pigments obtained from both the brown form and the red form are shown in

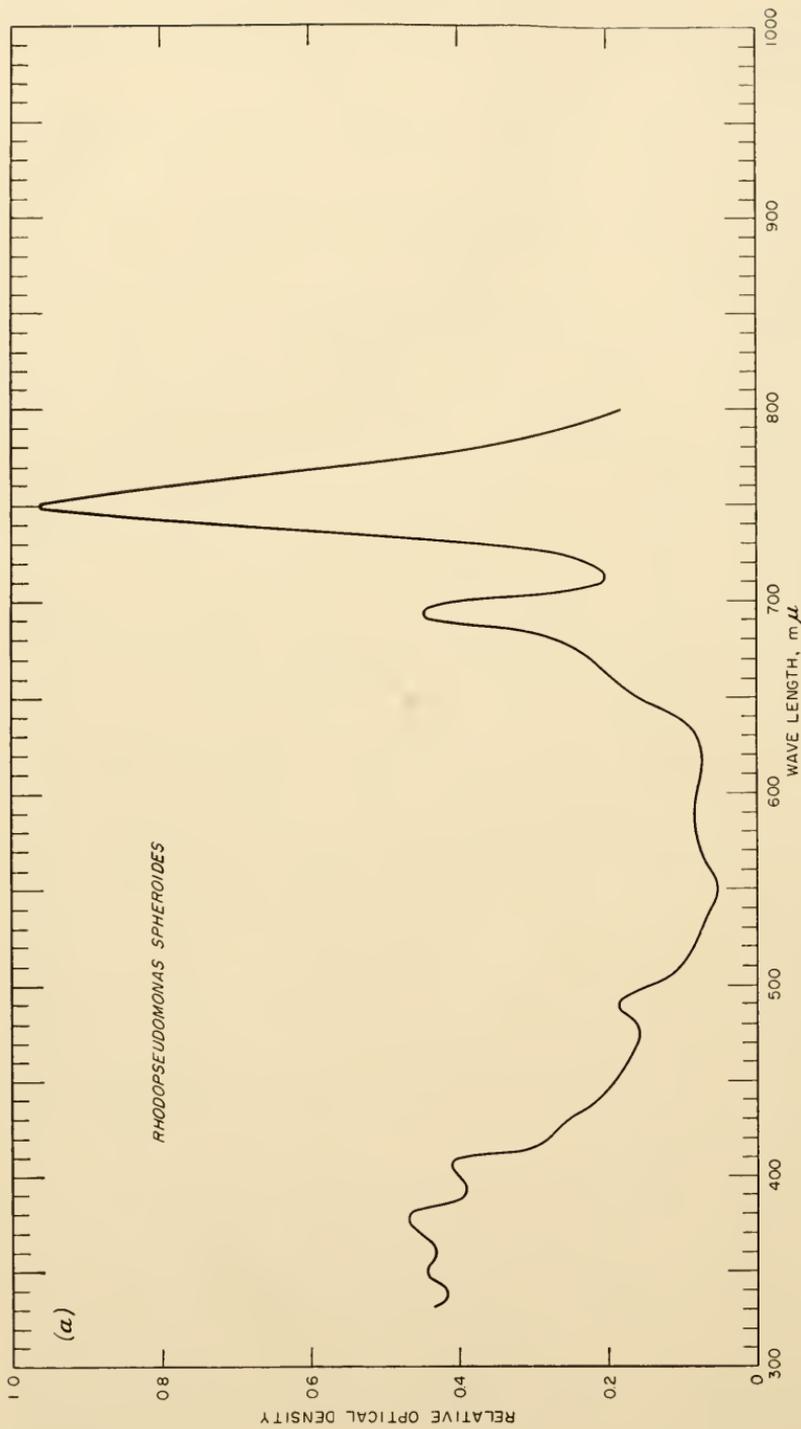


FIG. 6-12. The absorption spectra of the cell contents of various purple bacteria in saturated urea solution after cell disruption by supersonic vibration. (French, 1940a.) In part *d* (page 377) is shown also the action spectrum of photosynthesis in *Rhodospirillum rubrum* (French, 1937b) and the action spectrum of phototaxis (Manten, 1948). All the curves are brought to the same height at the 590-m $\mu$  peak of bacteriochlorophyll. (See the following three pages for parts *b* to *d* of Fig. 6-12.)

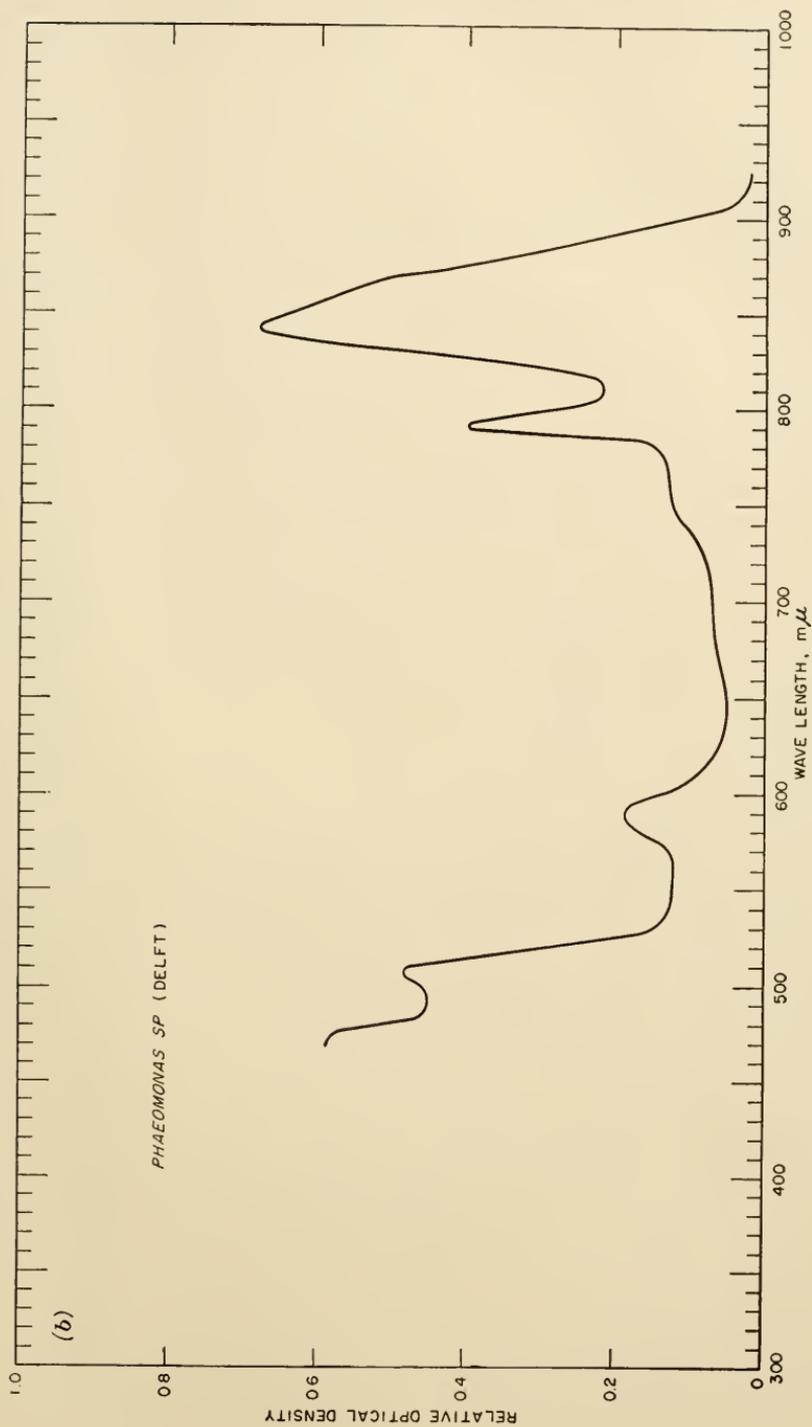


Fig. 6-12 (continued)

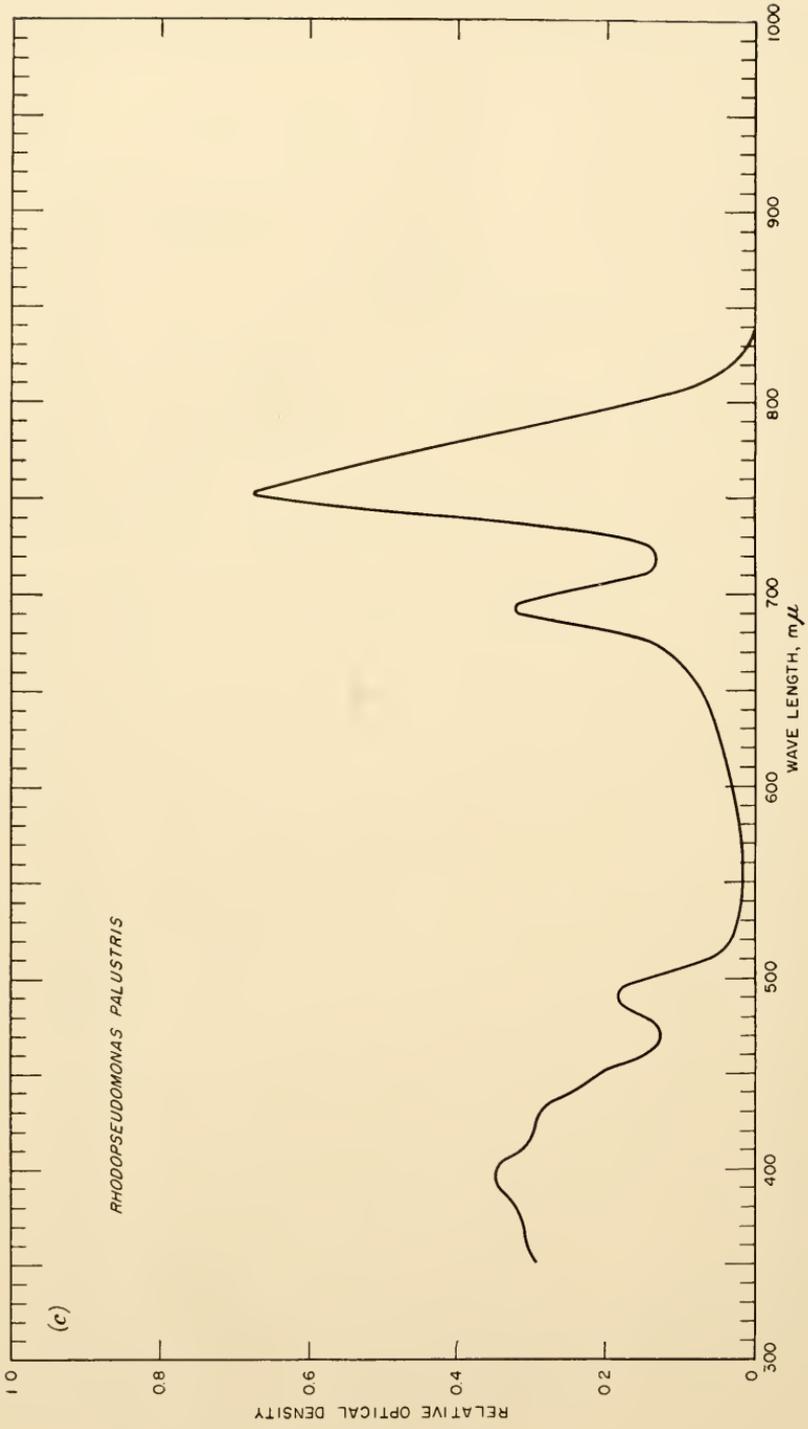


Fig. 6-12 (continued)

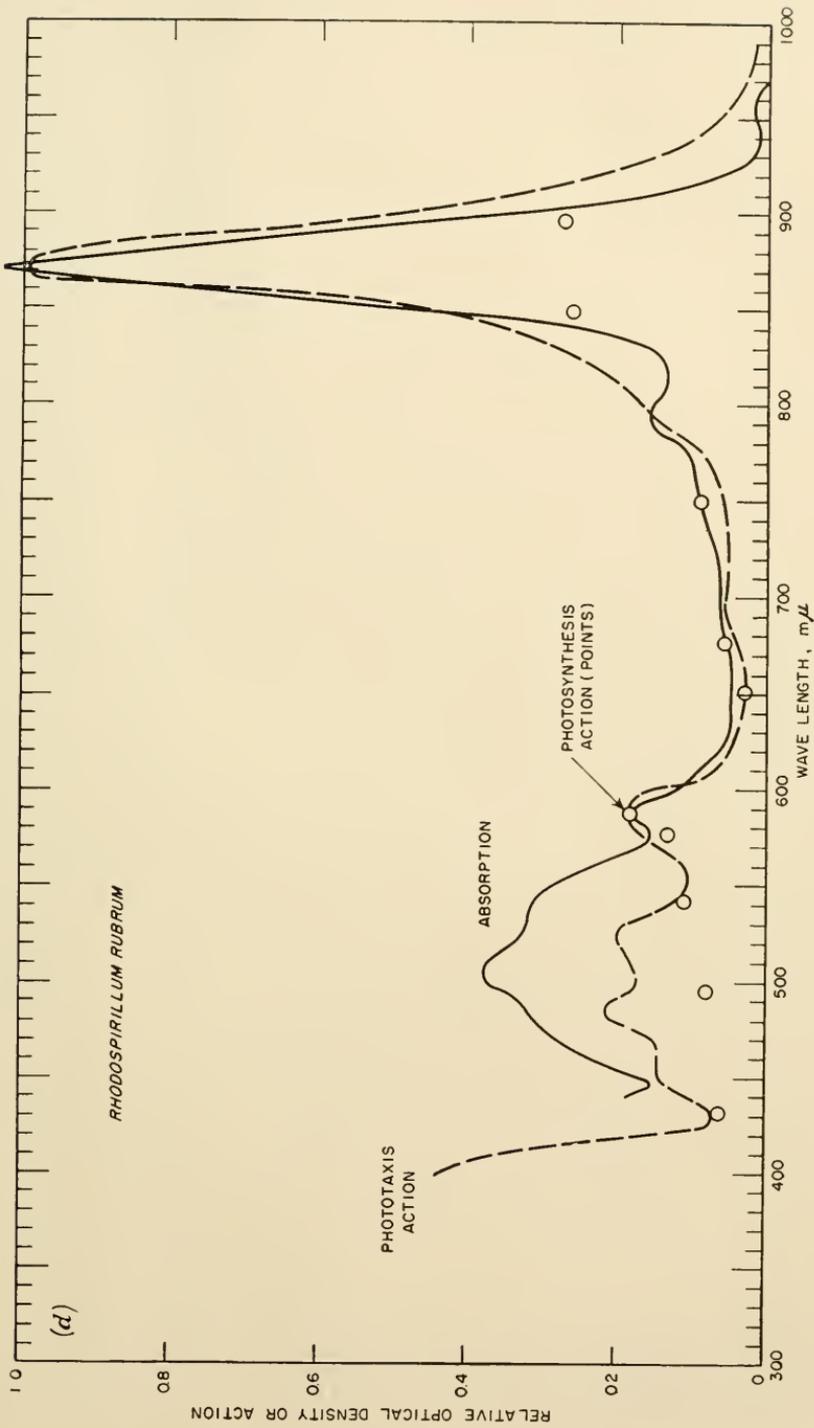


Fig. 6-12 (continued)

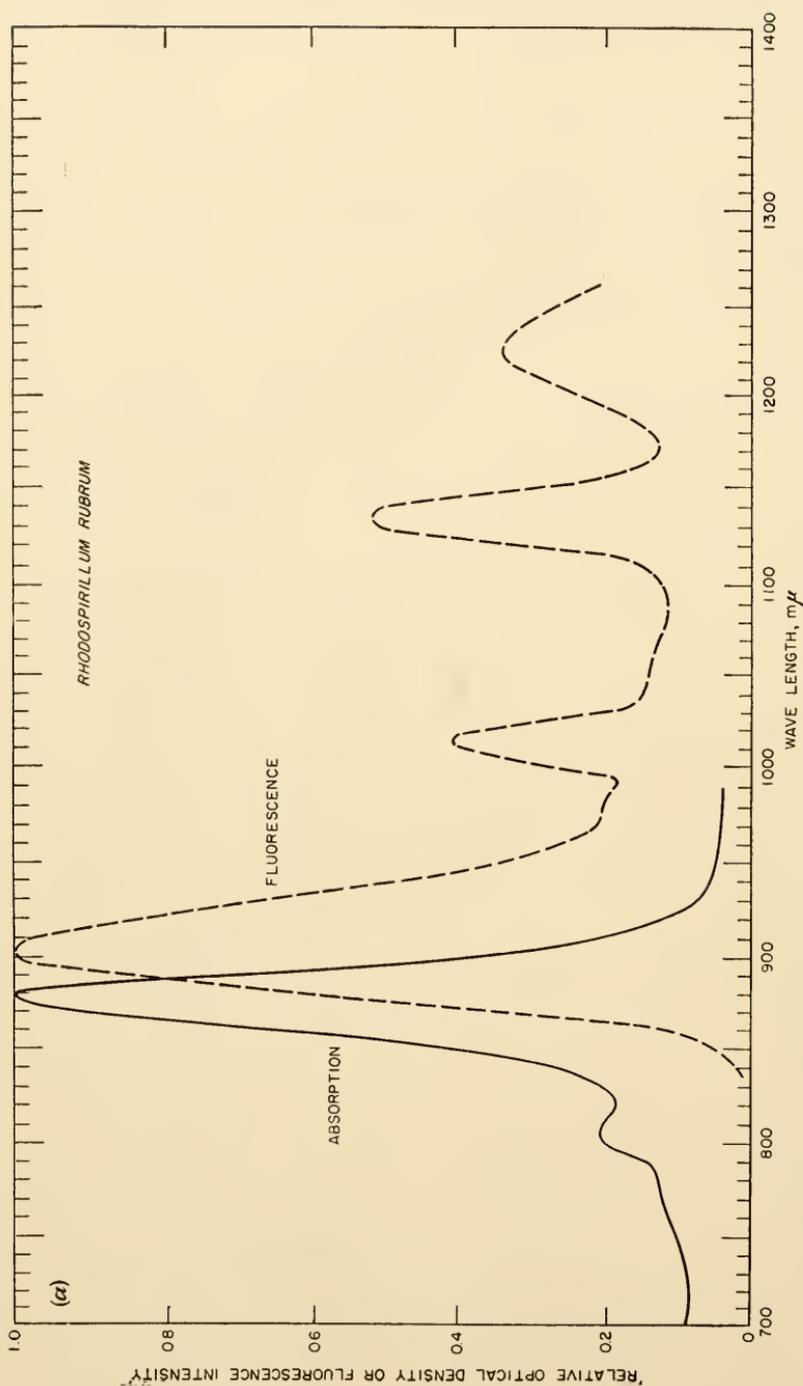


Fig. 6-13. (a) The absorption and fluorescence spectra of *Rhodospirillum rubrum* in the near infrared. (Duyens, 1951a, 1952.) (b) The absorption of pure bacteriochlorophyll in methanol (Manten, 1948) and its fluorescence in the same solvent (Vermeulen *et al.*, 1937). The shorter-wave-length fluorescence band has been found to be due to another pigment accompanying bacteriochlorophyll. (J. H. C. Smith and C. S. French, unpublished, 1954.) (c) The absorption and fluorescence spectra of *Chromatium* in the near infrared. (Duyens, 1951a, 1952.) (d) The absolute-absorption spectrum of bacteriohaeophytin in chloroform calculated from the equation  $K = \frac{1}{cd} \log \frac{I_0}{I}$  with  $c$  in milligrams per liter and  $d$  in centimeters. (French, 1940a.)

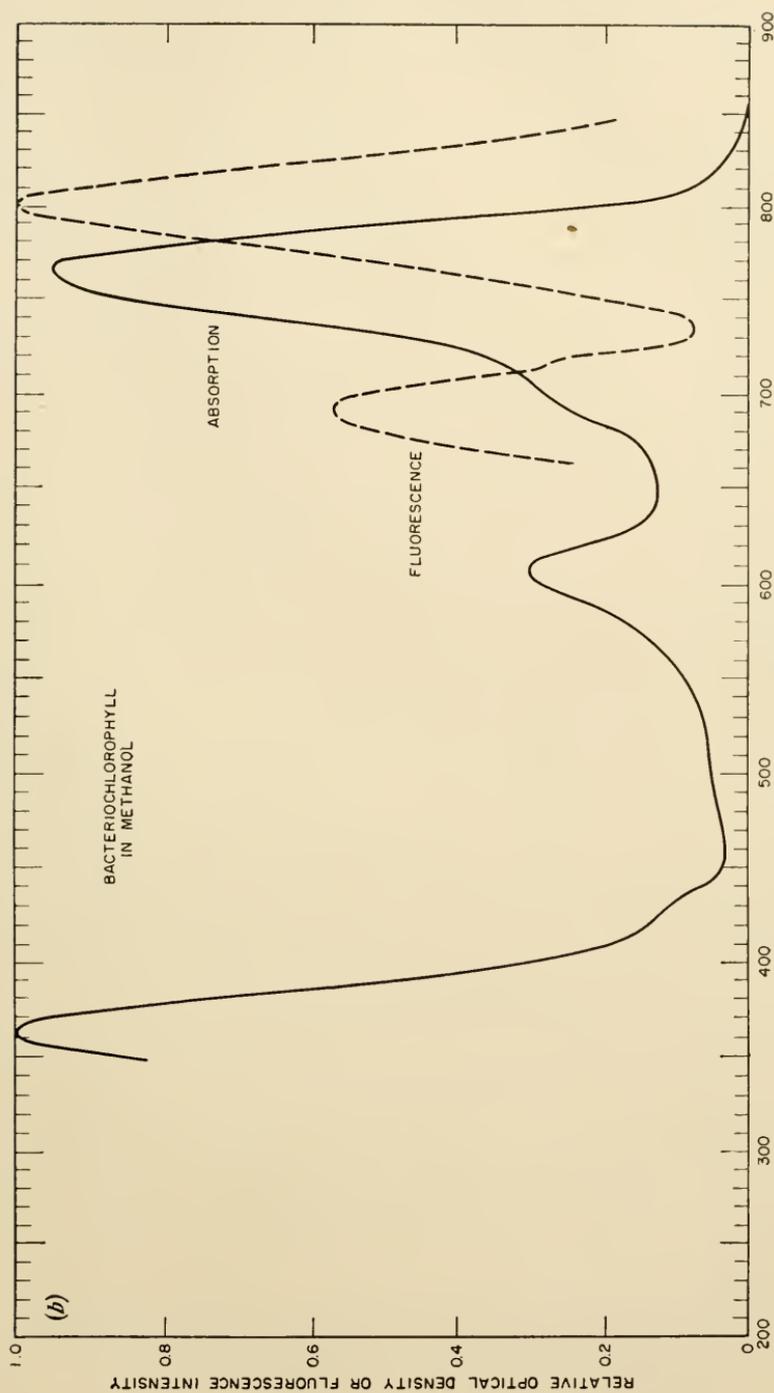


Fig. 6-13 (continued)

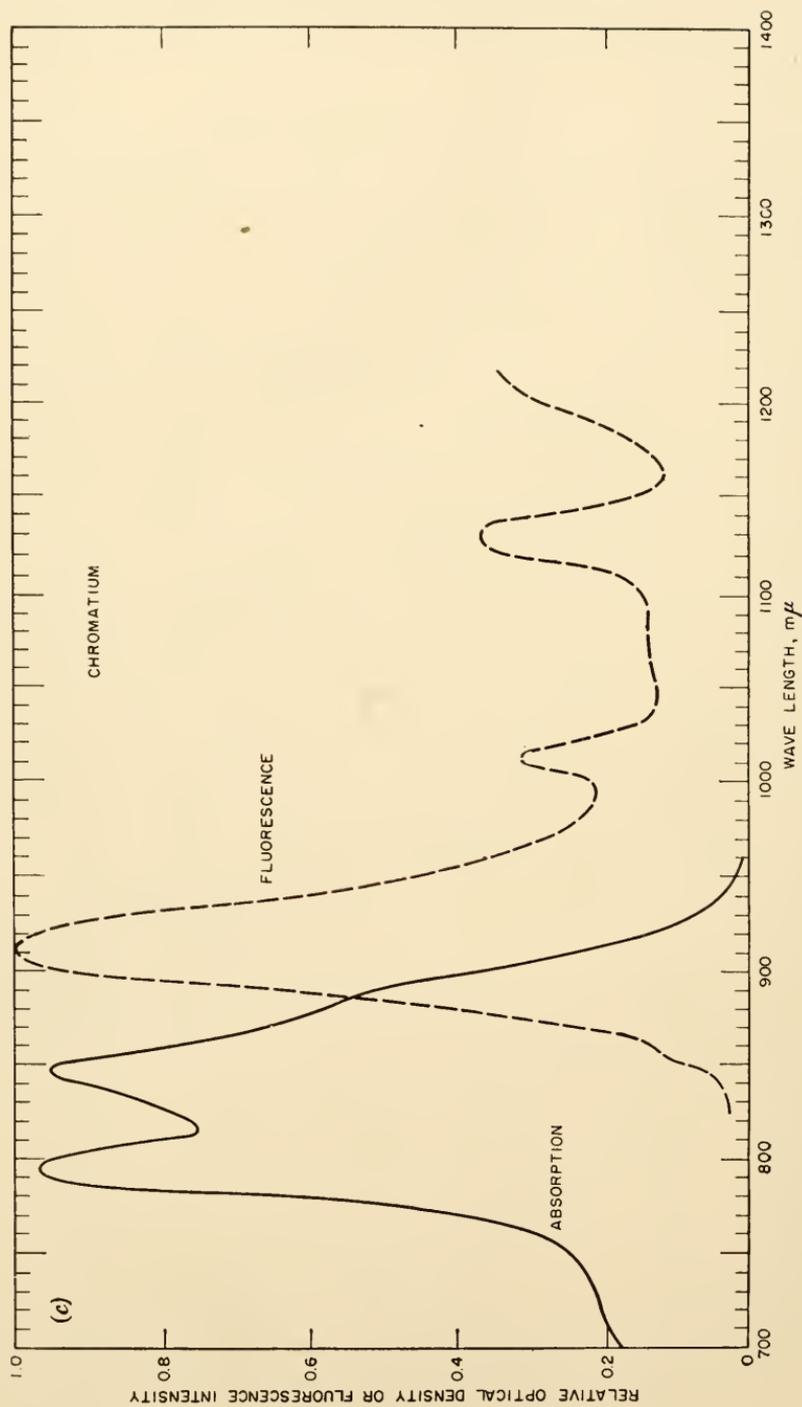


FIG. 6-13 (continued)

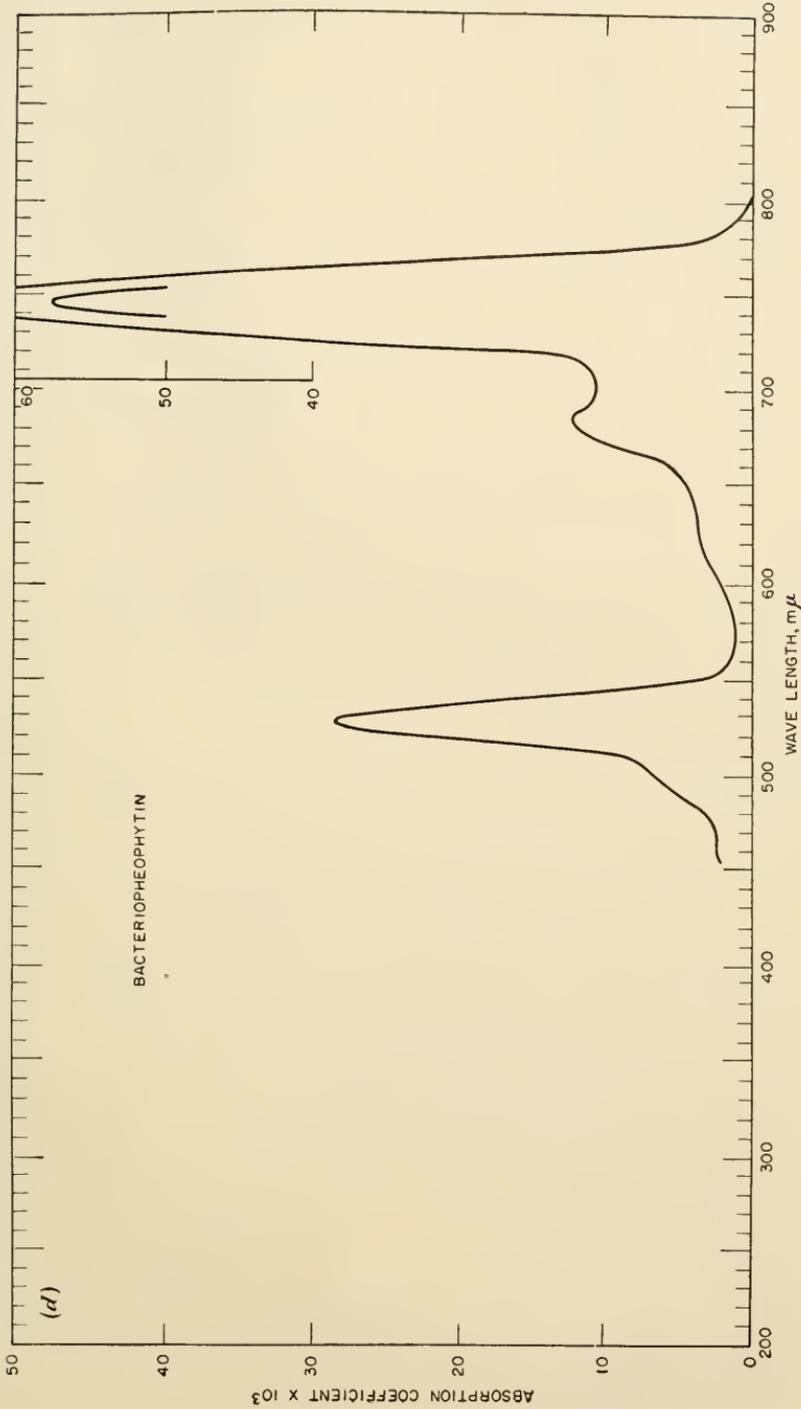


FIG. 6-13 (continued)

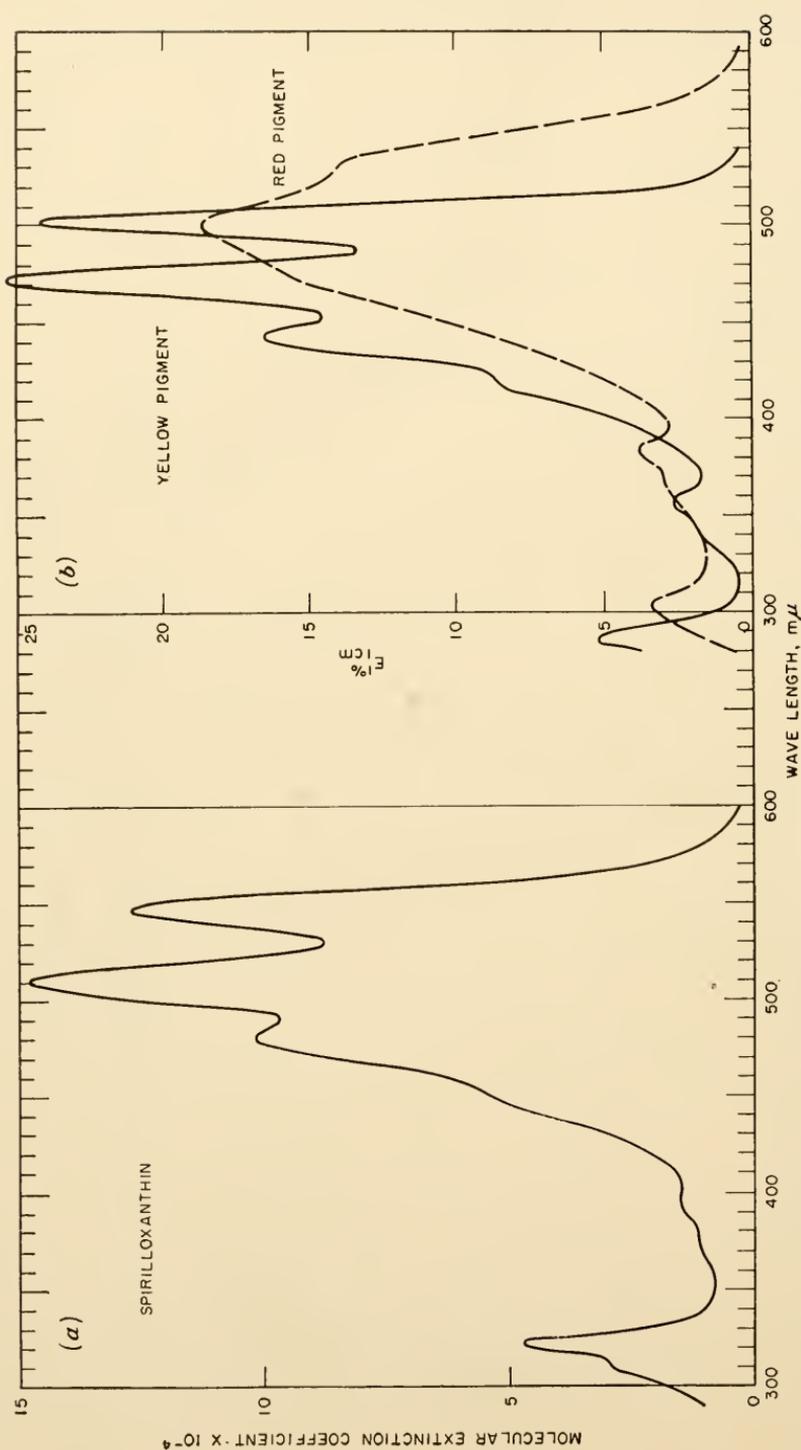


FIG. 6-14. The absorption spectra of several bacterial carotenoids in benzene. (a) Spirilloxanthin, fresh solution of the all-trans compound. (Polgár *et al.*, 1944.) (b) The yellow and the red unnamed pigments of *Rhodospseudomonas spheroides* (formerly *Streptococcus varians*), which produces the yellow pigment anaerobically and changes it into the red pigment in the presence of oxygen. (Van Niel, 1947.)

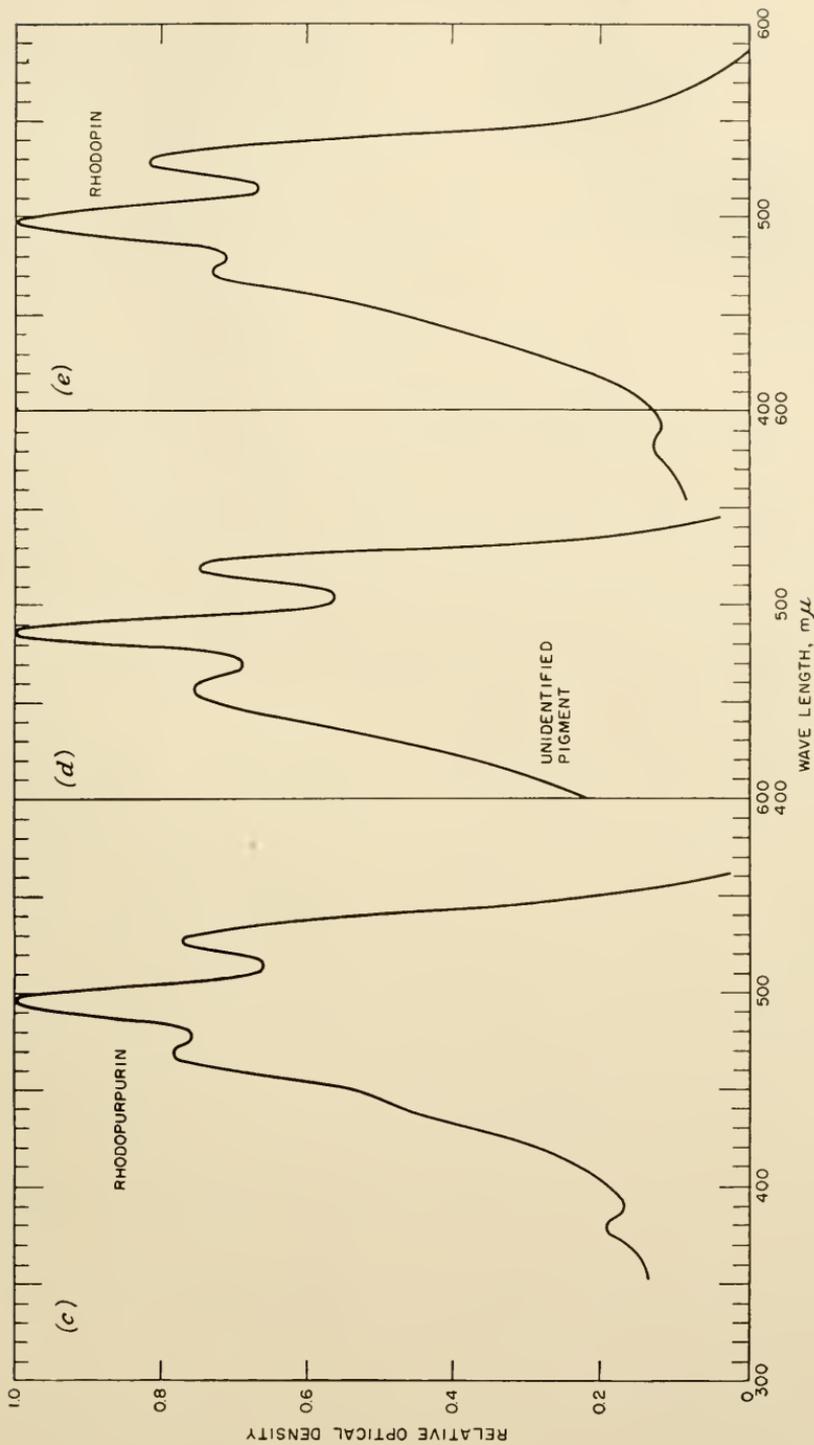


FIG. 6-14c, d, e. The pigments other than spirilloxanthin found in *Rhodospirillum rubrum*. (Mantou, 1948.)

Fig. 6-14b. The color change of the live bacteria was found by Van Niel (1947) to be due to the conversion of the yellow pigment, which gives the bacteria a brownish appearance, to a red pigment upon exposure to oxygen. The total carotenoid content remained constant during this conversion.

The purple bacteria are unusually valuable organisms for studies of the absorption spectra of pigments in the natural state because of the wide separation of the bands of the two different classes of pigments. Such measurements could well be used in the interpretation of the state of photosynthetic pigments in their functional environment.

#### ACTION SPECTRA OF PHOTOSYNTHESIS, PHOTOTAXIS, AND FLUORESCENCE EXCITATION IN PURPLE BACTERIA

The photosynthesis and growth of purple bacteria in projected spectra was found by Buder (1919) to take place at the absorption bands of bacteriochlorophyll and also at the bands due to carotenoid pigments. French's measurements (1937b) of photosynthesis of *Rhodospirillum rubrum*, however, showed that light absorbed by the bacteriochlorophyll was more effectively used than those wave lengths in the region of carotenoid absorption. It was therefore concluded that photosynthesis was done by bacteriochlorophyll but not by the bacterial carotenoids. These data are shown in Fig. 6-12d and compared with the absorption spectrum of an aqueous urea extract of these bacteria. Recent work on the action spectra for phototaxis and on photosynthesis and fluorescence excitation in this species by Manten (1948) and Thomas (1950) has cleared up the previous discrepancy. It has now been found that, although the light of wave lengths in the carotenoid absorption region is less effective than that absorbed by bacteriochlorophyll, there are very definite action peaks in this region which must be taken to indicate the participation of a small fraction of the carotenoids. The predominant carotenoid appears inactive, but others, present in lower concentration, are certainly effective. These results emphasize the need for measurements at many closely spaced wave lengths in the determination of action spectra. Figure 6-12d also shows Manten's action spectrum for phototaxis. The broad bands used for the photosynthesis measurements in the infrared account for the deviation of these two points from Manten's phototaxis curve. Manten concludes: "Thus it is about certain that in addition to bacteriochlorophyll, a carotenoid less abundant than spirilloxanthin and similar to rhodopin or rhodopurpurin is responsible for the absorption of the phototactically active light."

The photosynthesis measurements in *Rhodospirillum rubrum* by Thomas (1950) also show the carotenoid peaks in the action spectrum. In Fig. 6-15a we have plotted these data, as well as part of the phototaxis and absorption curves, on a larger scale, bringing all curves together at

the 590-m $\mu$  bacteriochlorophyll peak. It is clear that the spirilloxanthin that causes most of the carotenoid absorption in *Rhodospirillum rubrum* is not the active carotenoid, since the absorption bands and the action peaks do not agree. These two action spectra do, however, rather closely match the action spectra for phototaxis and for fluorescence excitation of *Chromatium* shown in Fig. 6-15b. In *Chromatium* the main carotenoid absorption peaks match those of the action spectra in position but not in height.

*Transfer of Energy from Bacterial Carotenoids to Bacteriochlorophyll as a Step in Photosynthesis.* We have seen in Figs. 6-12d and 15a and c that photosynthesis and phototaxis in purple bacteria are carried out not only by light absorbed by the bacteriochlorophyll itself but also by light absorbed by some of the carotenoids that are present in small amounts. That the energy absorbed by carotenoids is transferred to bacteriochlorophyll is indicated by the close match between the action spectrum for the excitation of bacteriochlorophyll fluorescence in Fig. 6-15c and that for phototaxis in the same bacteria, *Chromatium*. Quantitative measurements of fluorescence in *Rhodospirillum rubrum*, also by Duysens (personal communication, 1950), indicate that the carotenoid activity in this species also takes place by transfer of energy to the bacteriochlorophyll.

In addition to energy transfer from carotenoids to bacteriochlorophyll, a transfer from one type of bacteriochlorophyll molecule to another has been found by Duysens. This may be analogous to the transfer from chlorophyll b to a in green plants (Duysens, 1951a) and in solutions (Watson and Livingston, 1950). In Fig. 6-13a and c are presented the fluorescence spectra and the near-infrared absorption spectra of *Rhodospirillum* and of *Chromatium*.

In *Rhodospirillum* one type of bacteriochlorophyll occurs with an absorption peak at 890 m $\mu$ ; in *Chromatium* three types occur which have absorption maxima at 800, 855, and 890 m $\mu$ . The presence in *Chromatium* of the type with the longest-wave-length absorption peak is indicated by the hump at 890 m $\mu$  in the absorption spectrum. Quoting Duysens (1951a): "The fluorescence spectra of *Chromatium* and *Rhodospirillum rubrum* are similar, so probably also the absorption spectra of the emitting molecules are similar. The absorption spectrum of *Chromatium* might thus be analyzed in three spectra similar to the absorption spectrum of *Rhodospirillum rubrum*; only the molecules with absorption peak at 890 m $\mu$  show fluorescence. Upon heating, these molecules disappear, as shown by the absence of the hump at 890 m $\mu$  in the heated autolysate [Fig. 6-15b, d], and a new fluorescence peak occurs, probably from the molecules with an absorption peak at 855 m $\mu$ . This experiment suggests that in the presence of the molecules with an absorption peak at 890 m $\mu$ , transfer of energy occurs from the 855-m $\mu$  peak to the 890-m $\mu$  peak."

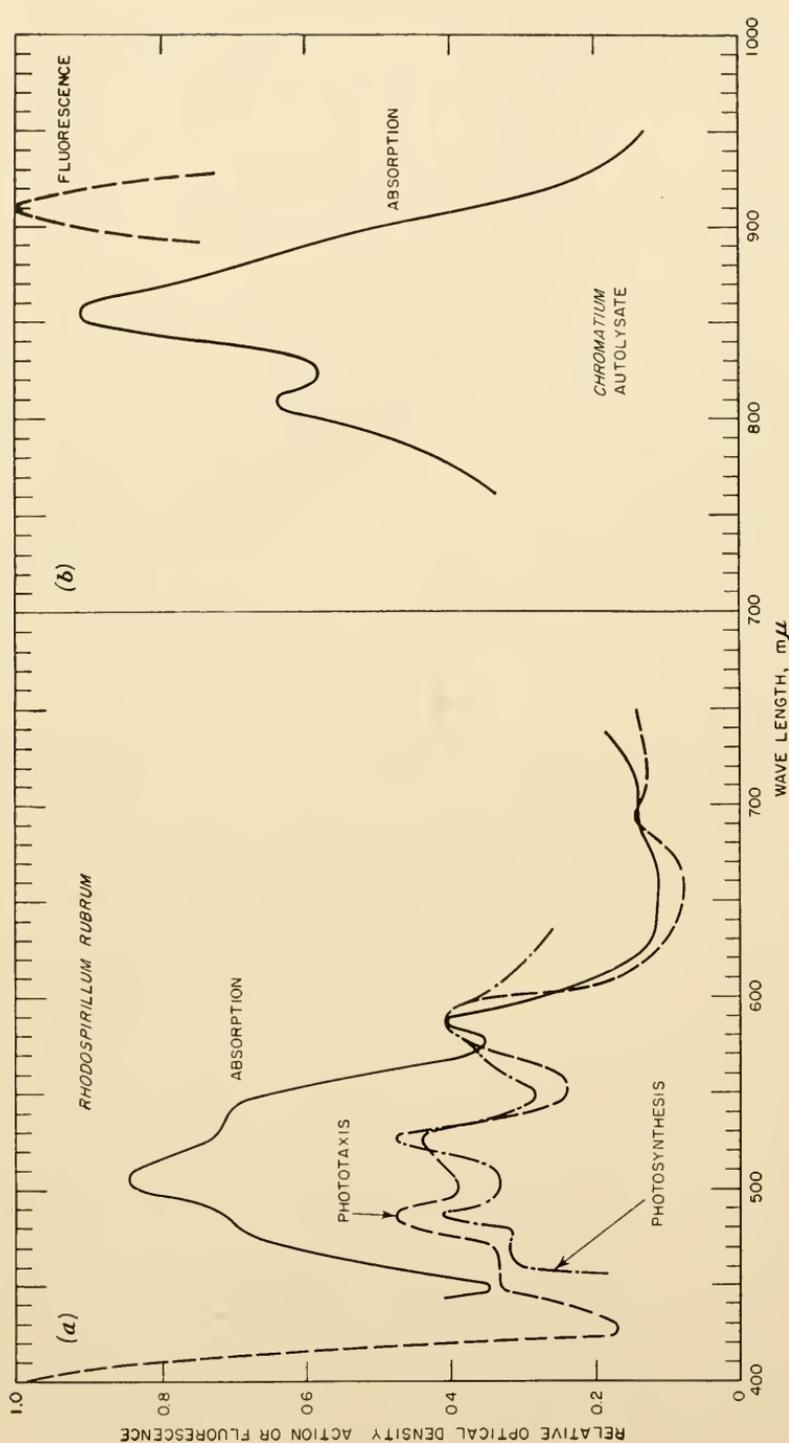


FIG. 6-15. (a) The absorption spectrum of *Rhodospirillum rubrum* in the visible (French, 1940a) as compared with the action spectra of phototaxis (Manten, 1948) and photosynthesis (Thomas, 1950). The curves were all brought together at the 590-m $\mu$  bacteriochlorophyll peak. The carotenoid action peaks do not appear in the absorption spectrum and hence must be due to a pigment present in much lower concentration than the spirilloxanthin which causes most of the absorption between 450 and 550 m $\mu$ . (b, d) Heating an autolysate of *Chromatium* destroys the 890-m $\mu$  component of the absorption spectrum. The fluorescence spectrum of the cells and of the autolysate

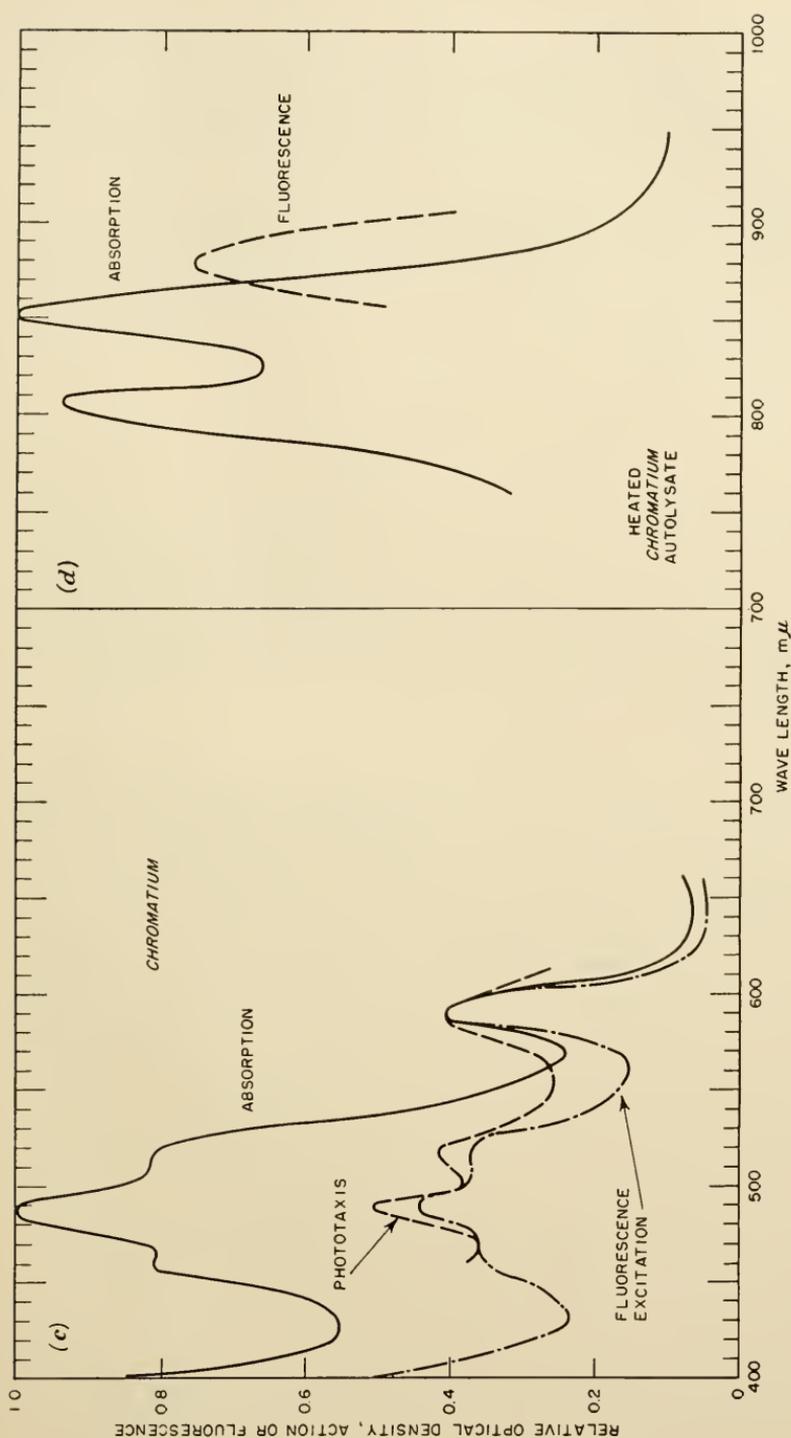


Fig. 6-15 (continued)

are due to emission by the part of the system which absorbs at  $890\text{ m}\mu$ . In the heated autolysate the fluorescence spectrum is associated with the system absorbing at  $855\text{ m}\mu$ . (Dyssen, 1951a.) (c) The absorption spectrum of *Chromatium* in the visible as compared with the spectra for phototaxis and for the excitation of bacteriochlorophyll fluorescence. (Dyssen, 1951b.) In *Chromatium* the carotenoid absorption peaks agree in position but not in height with the action peaks. The fluorescence excitation spectrum shows the transfer of energy from a carotenoid to bacteriochlorophyll.

Furthermore in *Chromatium* light quanta absorbed by the 800-m $\mu$  peak were found to bring about a strong fluorescence of the molecules with the 890-m $\mu$  peak, as did the quanta absorbed by these molecules themselves. Therefore a complete transfer of energy takes place from the molecules with the 800-m $\mu$  peak to those with the 890-m $\mu$  peak. It thus appears that, although several different compounds may absorb energy, the photosynthesis is carried out by a single type of bacteriochlorophyll protein complex. The situation here is strikingly similar to that of the blue-green and red algae, in which only chlorophyll a appears to participate directly in photosynthesis, whereas the energy absorbed by the other pigments is utilized by transfer to chlorophyll a.

### CONCLUDING REMARKS

In looking at the present state of the field covered here, two points stand out from the mass of accumulated data. One of these is the great inadequacy of the available information as to the absorption spectra of the individual pigments in living cells. The other is the indication that present spectroscopic data support the conclusion that all other pigments that absorb light used in photosynthesis act by transferring the energy to chlorophyll.

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## Chlorophyll Formation and Accumulation in Plants

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*Description of the naturally occurring process. Precursors of chlorophyll. Properties of protochlorophyll: Chemical nature of protochlorophyll—Physical properties of protochlorophyll—Physiological properties of protochlorophyll. Effect of temperature on chlorophyll formation and accumulation. Influence of the ambient atmosphere on the formation and accumulation of chlorophyll. Influence of nutrition on the formation and accumulation of chlorophyll. Chlorophyll formation in the dark. Use of chlorophyll mutants. Mechanism of chlorophyll biosynthesis. References.*

In photosynthetic organisms, seven types of chlorophyll have been identified: chlorophylls a, b, c, d, and e, bacteriochlorophyll, and bacterioviridin. The distribution of these pigments has been summarized by Strain (1949, 1951). Chlorophyll a is the most widely distributed of these pigments. It is "common to all autotrophic organisms except the pigmented bacteria" (Strain, 1949); chlorophyll b accompanies chlorophyll a in the higher plants and in some algae; and chlorophylls c, d, and e appear only in algae and associated with chlorophyll a. Bacteriochlorophyll is the chlorophyll of a number of strains of purple and brown bacteria (Van Niel and Arnold, 1938), and bacterioviridin, of the green bacteria (Metzner, 1922; cf. French and Young, Chap. 6, this volume).

Unfortunately the biosynthesis of only chlorophylls a and b has been studied in detail. Of necessity, therefore, this article will be devoted solely to a review of the development of these chlorophylls. Although these chlorophylls are found in organs other than the chief photosynthetic organs of plants, for example, in stems, flowers, seeds, roots, and tubers, space will not permit a discussion of the chlorophyll-forming process in these organs. For information regarding this aspect of the subject, the reader is referred to articles by Lubimenko (1926) and by Larsen (1949, 1950).

As an introduction to the analysis of the process of chlorophyll formation and accumulation, we will first describe the naturally occurring process in the higher plants.

## 1. DESCRIPTION OF THE NATURALLY OCCURRING PROCESS

Seeds of almost all angiosperms germinated in complete darkness produce seedlings that contain no chlorophyll. These seedlings become green when brought into light of proper intensity and wave length and under favorable conditions of temperature, nutrition, and ambient atmosphere. As is well known, the green color is attributable to chlorophyll.

Spectroscopic examination of extracts of unilluminated and briefly illuminated dark-grown seedlings reveals differences in the spectral

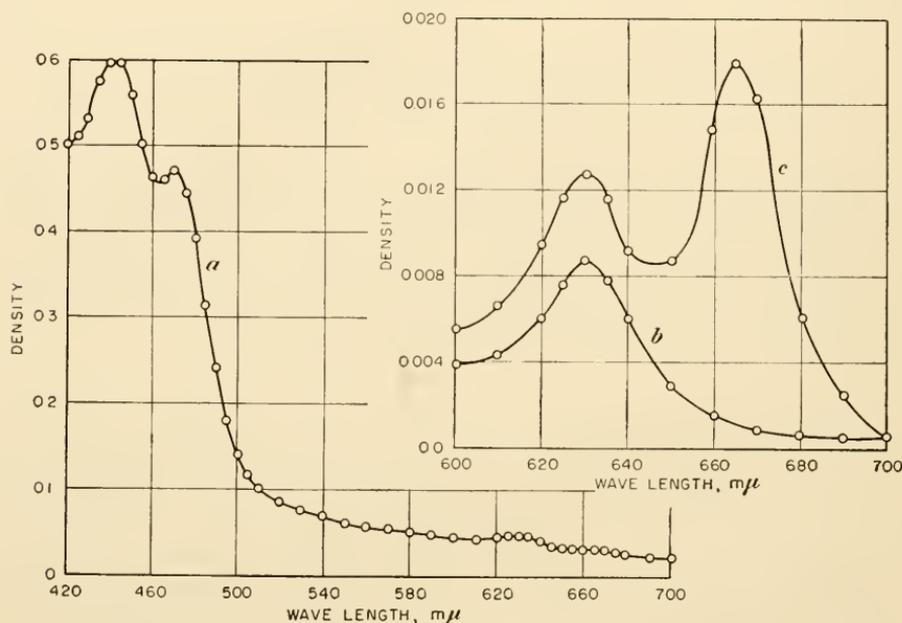


FIG. 7-1. Absorption spectra of methyl alcoholic extracts of dark-grown oat seedlings: curve *a*, from unilluminated seedlings, showing the carotenoid peaks at 440 and 470  $m\mu$ ; curve *b*, long-wave-length region of curve *a* enlarged so as to show the absorption maximum of protochlorophyll at 630  $m\mu$ ; curve *c*, from illuminated seedlings, showing the chlorophyll absorption maximum at 665  $m\mu$ . (Frank, 1946.)

absorption of the extracts. In Fig. 7-1 are shown the absorption spectra of methanol extracts of dark-grown oat seedlings. The extract of unilluminated seedlings, curve *a*, shows marked absorption in the violet end of the spectrum owing to the presence of carotenoids and a very small absorption maximum at about 630  $m\mu$ , characteristic of protochlorophyll. On an enlargement of the scale, curve *b*, this absorption band is emphasized. After the leaves are illuminated, a new absorption band appears in the extract at 665  $m\mu$ , curve *c*, which is attributable to chlorophyll *a*. The lesser absorption band of curve *c* at about 630  $m\mu$ , although in the same position as the absorption band of protochlorophyll, is also largely due to chlorophyll *a*.

A similar set of curves for ether extracts of barley seedlings is shown in Fig. 7-2. The leaves had been illuminated for periods of 0, 2, 24, 47, and 71 hr before extraction. In the extract of the unilluminated leaves the strong absorption of the carotenoids beyond 480  $m\mu$  and of protochlorophyll at 625  $m\mu$  (see inset) is apparent. Illumination of the leaves

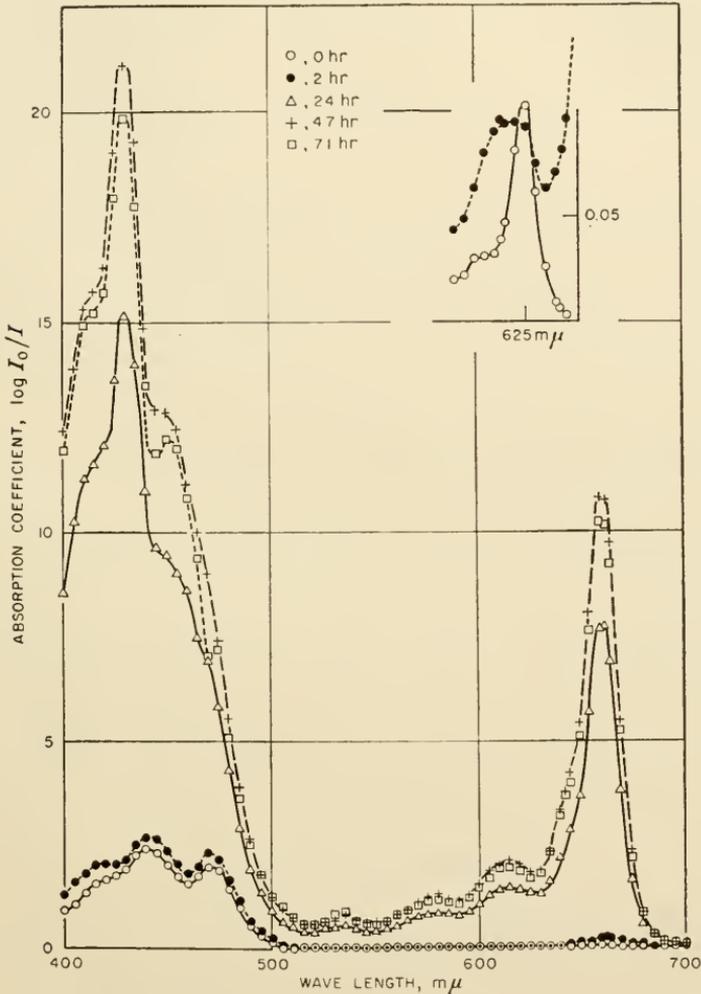


FIG. 7-2. Absorption coefficients of ether extracts of 50 g of barley leaves greened for different periods of time. (Smith, 1947.)

for 2 hr produces enough chlorophyll to be discernible in the extracts (see also in the inset) and causes the disappearance of the protochlorophyll spectrum. Longer illumination periods, up to 47 hr, increase the chlorophyll content greatly. Further illumination, however, produces no additional chlorophyll. The cessation of chlorophyll accumulation after

long periods of illumination is characteristic of the chlorophyll-forming process.

Short periods of illumination produce only chlorophyll a, but continued irradiation produces chlorophyll b as well. Once chlorophyll b has appeared, the two chlorophylls increase in constant proportion to one another (see Fig. 7-18).

The presence in dark-grown leaves of a pigment, protochlorophyll, whose rapid disappearance on illumination is accompanied by the simultaneous appearance of chlorophyll, suggests that this pigment is the immediate chlorophyll precursor, about the reality of which there has been much controversy.

In contrast to the angiosperms, which form chlorophyll only in the light, many other classes of plants form chlorophyll without light. When cultivated in the dark, many algae grow and retain their greenness, and some ferns put out new fronds that are green. But perhaps the most interesting plants possessing this property are the conifers, which, when grown from seed in the dark, produce seedlings with intensely green cotyledons.

Different groups of plants produce different chlorophylls and different relative proportions of the chlorophylls. There are genetic mutants that contain no chlorophyll, only a little chlorophyll, chlorophyll distributed in bizarre patterns, or chlorophyll-like compounds but no chlorophyll. An understanding of the causes of these variations would go far toward clarifying the mechanisms of chlorophyll formation and chlorophyll accumulation.

But analysis of all these aspects of chlorophyll formation and accumulation is beyond the scope of this review even if sufficient facts were available for such an analysis. Therefore only such selected aspects will be presented as will document and summarize the present concepts concerning the biosynthesis and accumulation of chlorophyll.

## 2. PRECURSORS OF CHLOROPHYLL

Inasmuch as we are endeavoring to understand the path of biosynthesis of chlorophyll, our attention naturally turns to the precursors of chlorophyll, especially to the immediate precursor of chlorophyll. The existence of such a specific precursor was postulated by Sachs as early as 1859.

Ideas concerning the formation of chlorophyll have centered around the concepts concerning the genesis, nature, and transformations of the chlorophyll precursor. These ideas have taken many forms and have been epitomized in the names given to this substance. The chief names applied to this precursor, along with a concise statement of the properties implied by each name, are presented here.

1. Leukophyll. Sachs (1859) applied the name "leukophyll" to what

he supposed was a colorless chromogen which is distributed in the protoplasm of leaves and which is transformed to chlorophyll by an oxidation analogous to the oxidation of indigo white to indigo.

2. Chlorophor. Chlorophor is the name given to the outer layer of chloroplasts by Boehm (1856, 1859) from which chlorophyll is formed and to which it is attached.

3. Carotenoid pigments. Kraus (1872) assumed that chlorophyll was formed at the expense of the yellow pigments present in etiolated leaves.

4. Etiolin. A pigment mixture extracted from supposedly etiolated leaves was called "etiolin" by Pringsheim (1874). Its function as a chlorophyll precursor was enunciated by Wiesner (1877). Concepts concerning its properties underwent continual change in order to keep pace with the advance in knowledge of the chlorophyll-forming process (Mikosch and Stöhr, 1880; Reinke, 1893; Greilach, 1904). Monteverde (1893-1894) and Timiriazeff (1903) clearly recognized it as a mixture of chlorophyll, protochlorophyll, carotene, and xanthophyll.

5. Protophyllin. Protophyllin was obtained artificially by Timiriazeff (1885, 1886a,b, 1903) as a nearly colorless reduction product of chlorophyll. It was readily oxidized by air to give a green color. Because the color change produced by its oxidation was similar to the color change observed in the greening of etiolated leaves, protophyllin was assigned the role of chlorophyll precursor in leaves.

6. Protochlorophyll. From his experiments on etiolated leaves, Monteverde (1893-1894) came to the conclusion that such leaves contained a pigment that was transformed to chlorophyll by the action of light. This substance he extracted from etiolated leaves with alcohol and called "protochlorophyll."

7. Chlorophyllogen. Because the positions of the spectral absorption bands of protochlorophyll extracted from pumpkin inner seed coats did not agree with those of the pigment in the plant tissue, Monteverde and Lubimenko (1909, 1911) thought that the pigments were different and proposed the name "chlorophyllogen" for the pigment in the living tissue. In a later publication, Lubimenko (1927, p. 181) stated that the two terms "protochlorophyll" and "chlorophyllogen" were synonymous.

8. Chlorophyllin  $\delta$ . The term "chlorophyllin  $\delta$ " was used by Tswett (1907) to bring the chlorophyll-like pigment of etiolated leaves into his system of nomenclature for the chlorophylls.

9. Protochlorophylls a and b. Seybold and Egle (1939) and Seybold (1948-1949) have obtained evidence from the examination of pumpkin seed coats for the existence of immediate precursors of both chlorophylls a and b. They have called these precursors "protochlorophylls a and b."

Comparison of the actual and assigned properties of the precursor leaves no doubt but that the name best suited to designate the immediate precursor of chlorophyll is "protochlorophyll." There are objections

to all the other terms used in this connection. The precursor is a colored compound; therefore the name "leukophyll" is a misnomer. "Chlorophor" connotes a chlorophyll-carrying substance rather than a direct pigmented precursor. The carotenoid pigments cannot be, from what we now know, the direct and complete progenitor of chlorophyll. Protochlorophyllin turns green in the presence of air and the absence of light and does not possess spectroscopic properties that agree with those of the constituent of etiolated leaves which forms chlorophyll. "Chlorophyllin  $\delta$ " is a term that assumes the precursor to be a chlorophyll (chlorin structure), which from chemical evidence appears to be erroneous. The terms "protochlorophyll" and "chlorophyllogen," as they have been applied, connote properties and reactions that agree well with the properties and reactions of the chlorophyll precursor as we know it today. It might be more precise to replace the term "protochlorophyll" with "protochlorophyll a," but to postulate a perfected precursor for chlorophyll b in etiolated leaves goes beyond and even contradicts the evidence we have at present.

A distinction between the terms "protochlorophyll" and "chlorophyllogen" is called for. These two terms are not synonymous, as Lubimenko assumed, but they bear a close relation to each other, the same relation that extracted chlorophyll bears to chlorophyll in the leaf (cf. Lubimenko, 1928, pp. 89-90). Our present knowledge concerning chlorophyll indicates that its state in the chloroplast is different from what it is after extraction with organic solvents. To chlorophyll in its natural state various names have been applied, e.g., "chloroplastin," "photosynthin," "phylochlorin" (cf. Mackinney, 1940). The concept common to all these terms is that chlorophyll in the plant is combined with some carrier—this is the complete natural pigment. By extraction with organic solvents, chlorophyll is dissociated from the carrier and becomes dispersed in true solution. The same difference applies to protochlorophyll and chlorophyllogen: protochlorophyll is the extracted pigment; chlorophyllogen is the pigment combined with a carrier in the plant material.

It is evident that a term is needed to designate the complete pigment as it exists in its natural state and without prejudice as to the nature of its combination or function. For this concept we suggest the general term "holochrome"<sup>1</sup> (Gr. *holos*, whole, + *chroma*, color). Such a term has analogous usage in the terms "holoenzyme," "holocellulose," "holophytic," and "holozoic." The adjective would be "holochromatic." Thus we have chlorophyll holochrome to designate chlorophyll in its natural state and protochlorophyll holochrome to designate protochlorophyll in its natural state—and this is chlorophyllogen.

<sup>1</sup> The authors are indebted to Prof. Raymond D. Harriman, Department of Classics, Stanford University, for his helpful advice in selecting the terms "holochrome" and "holochromatic."

By analogy with the use of the term "chlorophyll," "protochlorophyll" will be used in this article to designate the precursor of chlorophyll except when it is necessary to distinguish explicitly between its natural and derived states.

### 3. PROPERTIES OF PROTOCHLOROPHYLL

The assumption that protochlorophyll is the immediate precursor of chlorophyll provides a starting point for the analysis of the greening process in leaves. Much of what is said subsequently in this article is directed toward the establishment of this hypothesis and an analysis of its implications.

#### 3-1. CHEMICAL NATURE OF PROTOCHLOROPHYLL

In order to understand the biosynthesis of chlorophyll, it is necessary to know the chemical relations that exist between it and its precursor. For this reason a résumé of the chemistry of protochlorophyll in its relation to chlorophyll will be given.

Protochlorophyll has been isolated in solid form from two sources: the inner seed coats of pumpkin seeds (Noack and Kiessling, 1929) and etiolated barley leaves (Koski and Smith, 1948). For the details of preparation, the original papers must be consulted.

Noack and Kiessling (1929) determined that the protochlorophylls from pumpkin seed coats and from etiolated leaves possess the same absorption spectra and the same basicity reactions. From these similarities they concluded that the protochlorophylls from the two sources are identical. Inasmuch as the content of protochlorophyll is greater in seed coats, they proceeded to carry out studies on the chemical structure of protochlorophyll from this source. They established that protochlorophyll is a complex magnesium compound from which magnesium can be removed with acid and reintroduced by means of the Grignard reagent. The magnesium-free compound they termed protopheophytin. They believed the pigment to be a diester of phytol and methanol, similar to chlorophyll. Later (1930), however, they questioned the presence of phytol, a question that subsequent investigators have also raised (Stoll and Wiedemann, 1938; Granick, 1950). They concluded that protochlorophyll was closely related to chlorophyll, because they could obtain the same product, protophytochlorintrimethyl ester [vinylchloroporphyrin- $e_6$  (Fischer and Stern, 1943, p. 323)], from protopheophytin and from pheophytin a.

Stoll and Wiedemann (1938) were the first to suggest a structural formula for protochlorophyll. They suggested that it was the magnesium complex of the phytol methyl diester of 2-vinylpheoporphyrin- $a_5$ .

The analytical and synthetic work of Fischer and his collaborators (Fischer, Oestreicher, and Albert, 1939; Fischer, Mittenzwei, and Oes-



gen atoms situated in positions 7 and 8 of ring IV. However, on theoretical grounds this structure has been called in question by Rabinowitch (1951, p. 623). Perhaps the same possibility exists for protochlorophyll which exists for chlorophyll, that the conjugated double bonds may not have fixed positions (cf. Smith, 1937, p. 500; Stoll and Wiedemann, 1938, p. 211).

Although the phytyl group has not been identified directly in protochlorophyll, indirect evidence points to its presence. The magnesium content of protochlorophyll from leaves, 2.73 per cent (Koski and Smith, 1948), agrees with that calculated from the phytyl-containing structural formula for protochlorophyll proposed by Fischer and Oestreicher (1939-1940).

The distribution of protochlorophyll and its derivatives between aqueous hydrochloric acid and ether [the hydrochloric acid number; see Fischer and Orth (1943, p. 601)] has been used to indicate the presence or absence of the phytyl group. By this test, Noack and Kiessling (1929, p. 16) found pumpkin-seed-coat protochlorophyll to color a 12 per cent hydrochloric acid solution, whereas chlorophyll a did not color a 22 per cent solution. This low value for protochlorophyll suggested the absence of the phytyl group. But Granick (1950) examined protochlorophyll prepared from etiolated barley leaves by the procedure of Koski and Smith (1948) and found a hydrochloric acid number of  $\sim 25$ . For protochlorophyll minus the phytyl group the value was  $\sim 11$ . Thus the presence of a phytyl group in undamaged protochlorophyll is indicated.

Attempts to show the presence of the phytyl group in protochlorophyll by means of the action of chlorophyllase have not been successful (Noack and Kiessling, 1929; Mayer, 1930). That chlorophyllase may act on protochlorophyll, however, is strongly indicated by the observations of Granick (1950).

Krasnovskii and Voinovskaya (1949)<sup>2</sup> claim that protochlorophyll is reduced photochemically in pyridine solution by ascorbic acid and that it can act as a photosensitizing agent for the transfer of hydrogen from ascorbic acid to saffranin T.

### 3-2. PHYSICAL PROPERTIES OF PROTOCHLOROPHYLL

*Spectral Absorption of Protochlorophyll.* Of all the characteristic physical properties possessed by protochlorophyll, its spectral absorption is perhaps the most useful. It has proved to be invaluable for identifying this pigment in various extracts, for determining it quantitatively, and for identifying it as the active light-absorbing agent for its own transformation to chlorophyll.

The absorption bands of protochlorophyll were first measured in

<sup>2</sup> The authors are indebted to Harold W. Milner for his translation of this article from the Russian.

extracts of etiolated leaves by Monteverde (1893-1894). The values reported by Monteverde for moderate concentrations of the pigment in alcohol are

E.A.—680; 640—620; 589—570; 535—E.A.  $m\mu$ .

A number of other workers have determined the positions of the absorption bands of protochlorophyll in crude extracts of the pigment from natural sources (Liro, 1908; Monteverde and Lubimenko, 1911; Lubimenko, 1928; Noack and Kiessling, 1929; Scharfnagel, 1931).

“Synthetic protochlorophyll” exhibits the following absorption bands (Fischer and Oestreicher, 1939-1940) in ether-pyridine solution:

628.3—617.1—599.5; 579.6—565.1—548.8; 463.0—E.A.  $m\mu$ .

Protochlorophyll isolated by chromatographic methods and dissolved in various solvents possesses absorption maxima at the wave lengths shown in Table 7-1. The measurements were made by means of Beckman spectrophotometers. The close agreement in the positions of the

TABLE 7-1. ABSORPTION MAXIMA OF PROTOCHLOROPHYLL

Source	Solvent	Absorption maxima, $m\mu$				Reference
		623	571	535	432	
Purified from etiolated barley leaves	Ether	623	571	535	432	Koski and Smith (1948)
	Methanol	629	578	...	434	
	Acetone	623	571	535	432	
Purified from seed coats of pumpkin	Ether	623	571	533	433	Krasnovskii and Voinovskaya (1949)
	Pyridine	633	588	550	453	

absorption maxima of ether solutions of protochlorophyll from etiolated leaves and from seed coats makes it reasonable to conclude that the pigments from the two sources are identical (cf. Fig. 7-5).

The positions of the absorption maxima of protochlorophyll in living tissues are difficult to observe directly, because only a small quantity of pigment is present in etiolated leaves and because the conversion to chlorophyll is usually very rapid when the tissues are brought into the light. In spite of these difficulties, attempts to measure the band positions have been made. The results show little consistency. The positions of the absorption bands of protochlorophyll, as reported by Liro (1908) for etiolated leaves treated in different ways, are as follows:

Etiolated leaves of *Sinapis* and *Helianthus* killed with alcohol vapor,

E.A.—690; 640—625; 589—580; 545—540  $m\mu$ ;

Etiolated leaves of 12 species of plants killed by freezing,

E.A.—690; 640—625; 588—580  $m\mu$ ;

Living etiolated leaves not too young,

E.A.—670; 640—620; 589—580; 545—540  $m\mu$ .

Because protochlorophyll occurs in much higher concentration and is transformed much more slowly in the inner seed coats of certain cucurbits than in etiolated leaves, it is easier to determine the positions of the absorption maxima in the seed coats. Accordingly Monteverde and Lubimenko (1911) have reported the following absorption bands in the seed coats of certain Cucurbitaceae seeds:

650—640 (620); 600—570; 550—530; 510—E.A.  $m\mu$ .

Krasnovskii and Voinovskaya (1949) have found the principal absorption band to lie between 650 and 645  $m\mu$  in pumpkin seed coats.

Monteverde and Lubimenko (1911) examined the seed coats of *Luffa*. The positions of the bands differed in the outer and inner seed coats:

Outer seed coat, 680—660; 610—590; 570—550; 510—480; 450—E.A.  $m\mu$ .

Inner seed coat, 650—640—620; 600—570; 550—530; 510—E.A.  $m\mu$ .

Extracts of the two gave the same absorption spectrum, which was characteristic of protochlorophyll. When heated, the color of the outer seed coats was changed from brown to green. Lubimenko (1928) compared the absorption bands of the *Luffa* seed coats with those of alcoholic extracts of the same:

Seed coats, 680—660; 610—590; 570—550; 510—480; 450—E.A.  $m\mu$ .

Alcoholic extracts, 640—620; 590—570; 540—525; 470—E.A.  $m\mu$ .

The fact that the positions of the absorption bands in the seed coats vary in different samples, whereas in the extracts of the same they agree with those of protochlorophyll, indicates that the different tissues contain different protochlorophyll holochromes.

The absorption curves for protochlorophyll in various solvents have been obtained by Rudolph (1934), Seybold and Egle (1939), Koski and Smith (1948), Seybold (1948–1949), and Krasnovskii and Voinovskaya (1949). Only the determinations of Koski and Smith are on an absolute-weight basis and for purified protochlorophyll from etiolated leaves (Fig. 7-4). The results of Seybold (1948–1949) purport to show the presence of protochlorophylls a and b in pumpkin seed coats. The existence of protochlorophyll b is questionable, since other investigators have failed to find it either in seed coats (Noack, 1934; Fischer and Oestreicher, 1939–1940; Krasnovskii and Voinovskaya, 1949) or in etiolated leaves (Koski and Smith, 1948).

The absorption curves for protochlorophyll in ether, acetone, and methanol are given in Fig. 7-4 (Koski and Smith, 1948). The protochlorophyll used was obtained from barley leaves. It was prepared in pure solid form and was weighed directly into the solvents for the absorption-spectra measurements.

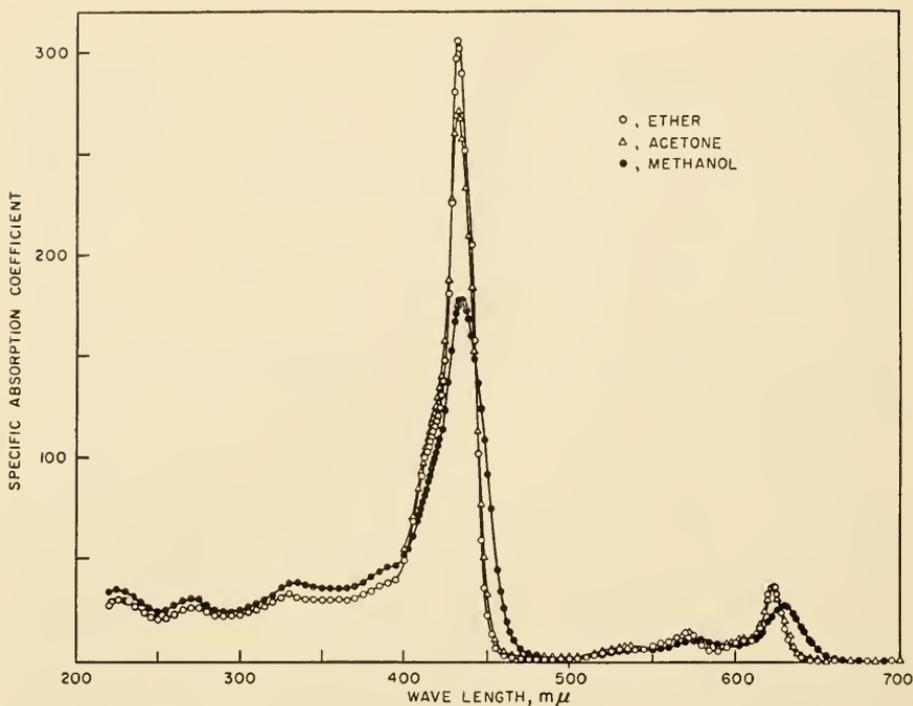


FIG. 7-4. The absorption spectra of precipitated and dried protochlorophyll dissolved in ether, acetone, and methanol. (Koski and Smith, 1948.)

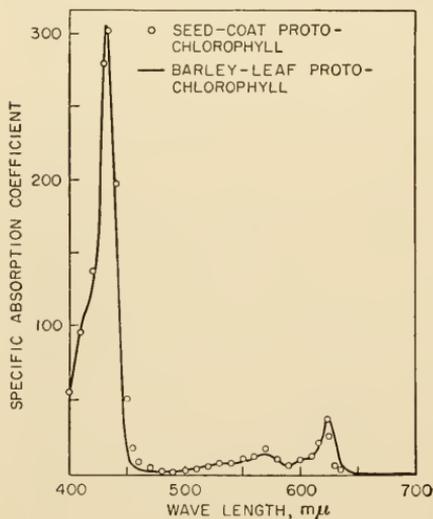


FIG. 7-5.

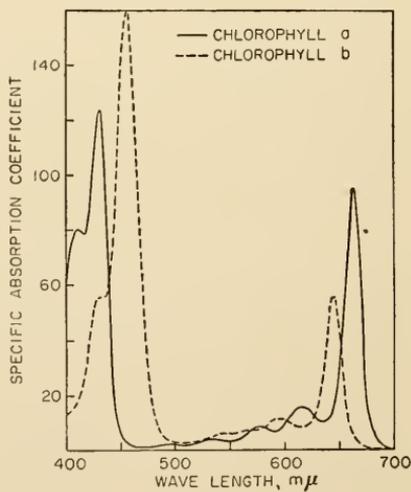


FIG. 7-6.

FIG. 7-5. Comparison of absorption spectra of barley-leaf protochlorophyll and pumpkin-seed-coat protochlorophyll in ether. The relative absorption values of seed-coat protochlorophyll (Krasnovskii and Voinovskaya, 1949) are compared with the absorption curve of barley-leaf protochlorophyll (Koski and Smith, 1948) after the value of the seed-coat protochlorophyll has been adjusted to the curve at 623  $m\mu$ .

FIG. 7-6. Absorption spectra of chromatographically purified chlorophylls a and b in ether. The chlorophyll content of the solutions was determined by magnesium analysis.

The absorption curves of protochlorophyll from pumpkin seed coats dissolved in ether and in pyridine have been determined by Krasnovskii and Voinovskaya (1949). The similarity of the curves for ether solutions of protochlorophyll from leaves and pumpkin seed coats is remarkable (Fig. 7-5).

For comparison, the absorption spectra of chlorophylls a and b dissolved in ether are given in Fig. 7-6. These measurements were made on chromatographically purified pigments, and the specific absorption coefficients calculated from the magnesium content of the solutions by converting them to actual concentrations of chlorophyll. The values agree reasonably well with those published by Comar and Zscheile (1942) and Mackinney (1941) (see also Zscheile *et al.*, 1942).

*Absorption Spectrum of Protopheophytin.*

In connection with studies on protochlorophyll, the absorption spectrum of protopheophytin has frequently been of use. Absorption curves of protopheophytin derived from protochlorophyll have been measured by Seybold (1948-1949) and by Koski (1949). The absorption curve for "synthetic" vinylpheoporpyrin-a<sub>5</sub> monomethyl ester in dioxane has been published by Granick (1950). Both Koski and Granick determined the absolute values of the specific

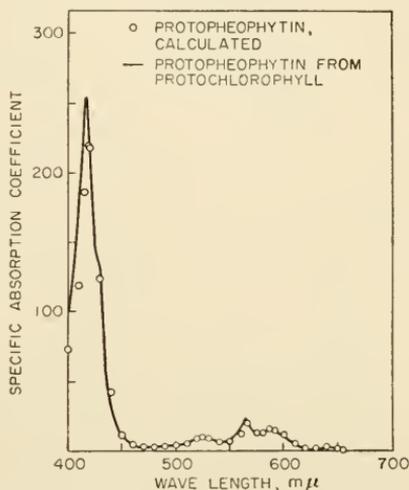


FIG. 7-7. Comparison of protopheophytin values calculated from the absorption measurements of Granick (1950) on "synthetic" vinylpheoporpyrin-a<sub>5</sub> methyl ester in dioxane with the absorption spectrum in ether of protopheophytin from protochlorophyll out of barley leaves. (Koski, 1949.)

absorption coefficients. A comparison of these two measurements is given in Fig. 7-7. The similarity of the values gives confirmatory evidence for the identity of the basic structures of the substances derived from chlorophyll a and from protochlorophyll.

The positions of the absorption maxima and the corresponding specific absorption coefficients for protopheophytin in ether solution are as follows (cf. Koski and Smith, 1948):

Absorption-maxima wave lengths, mμ.....	638	585	565	524	417
Specific absorption coefficients.....	2.5	17.3	25.1	11.7	256.6

*Fluorescence of Protochlorophyll.* Mikosch and Stöhr (1880) were probably the first to recognize the orange fluorescence of protochlorophyll. Since then, the fluorescence of this pigment has been measured by several

workers who have obtained very discordant results. The inconsistency may be due to errors arising from partial reabsorption of fluorescent light. The results are tabulated in Table 7-2.

The fluorescence band reported by Rothemund appears to be too far to the red when compared with the other values. A similar discrepancy may be noted for the fluorescence bands of chlorophyll measured in the same laboratory (cf. Rabinowitch, 1951, p. 744). The position of the fluorescence maximum in the corn leaf is independent of the wave length

TABLE 7-2. FLUORESCENCE BANDS OF PROTOCHLOROPHYLL

Protochlorophyll source	Medium	Wave length, $m\mu$	Reference
Seed coats.....	Ether	619-657	Noack and Kiessling (1929)
Etiolated leaves			
Wheat.....	Ether	626.5	Dhéré (1939)
Wheat.....	Methanol	643	Dhéré (1939)
Corn.....	Ether	645-665	Rothemund (1935)
Barley.....	Ether	629	V. M. K. Young (unpublished)
Corn.....	Living leaf	638	V. M. K. Young (unpublished)

of the exciting light and is unaffected by killing the leaf through hot-water immersion (V. M. K. Young, unpublished).

*Optical Activity.* The porphyrin nucleus of protochlorophyll is optically inactive. This places the seat of optical activity in chlorophyll a at the 7 or 8 position (Fischer, 1940).

### 3-3. PHYSIOLOGICAL PROPERTIES OF PROTOCHLOROPHYLL

The formation and transformation of protochlorophyll in the angiosperms will be discussed first.

*Formation of Protochlorophyll in Dark-grown Seedlings.* Since most seeds contain no protochlorophyll, this pigment must result from the germination process (Liro, 1908, pp. 21 ff.). The pigment is detectable after the appearance of the root. The production of protochlorophyll during the growth of dark-grown barley seedlings has been followed by Smith (1950-1951).

No. of days from planting.....	3	4	5	6	7	8	10
Protochlorophyll, $\mu\text{g}$ per 10 plants...	0.23	0.85	2.28	3.17	4.09	4.61	5.33

When plotted, these values give a typical sigmoid-shaped curve. As development of the seedlings proceeds, the protochlorophyll content increases slowly at first, then at an accelerated rate, and finally slows down as it approaches a limiting value. These results also confirm the observations of others that little if any protochlorophyll is present in the initial stages of germination.

The quantity of protochlorophyll formed in dark-grown seedlings is

affected by genetic factors. The protochlorophyll content of several chlorophyll-deficient mutants of corn was found to vary considerably in relation to the protochlorophyll content of their normal sibs, as reference

TABLE 7-3. COMPARISON OF VARIOUS DARK-GROWN CORN MUTANTS WITH THEIR DARK-GROWN NORMAL SIBS RELATIVE TO PROTOCHLOROPHYLL CONTENT (Koski, 1949.)

Corn mutant	Protochlorophyll content, mg/g dry weight		Percentage of normal
	Mutant	Normal	
<i>w</i> <sub>3</sub> .....	0.0411	0.0372	110.4
<i>v</i> <sub>2</sub> .....	0.0079	0.0358	22.1
<i>v</i> <sub>12</sub> .....	0.0027	0.0423	6.3
<i>v</i> <sub>4</sub> .....	0.0062	0.0327	18.9
<i>ij</i> .....	0.0051	0.0420	12.2
<i>g</i> <sub>1</sub> .....	0.0184	0.0265	69.3

to Table 7-3 shows. One result in this table which is very striking is the large protochlorophyll content of white seedling-3—an albino mutant of corn—which is greater than that of its normal sib. All the other chlorophyll-deficient mutants studied contained less protochlorophyll than their normal sibs.

*Photochemical Transformation of Protochlorophyll to Chlorophyll a.* Liro (1908) pointed out that greening and chlorophyll formation are clearly differentiated. Although greening is the result of chlorophyll formation, chlorophyll formation may take place without the plant becoming green. This section will be limited largely to a discussion of the formation of chlorophyll by the transformation of protochlorophyll in the living leaf.

When etiolated seedlings are illuminated, they first form only chlorophyll a (Seybold and Egle, 1938; Goodwin and Owens, 1947; Smith, 1949b; Blaauw-Jansen *et al.*, 1950; Seybold, 1948-1949). (This is evidence against the existence of protochlorophyll b in etiolated leaves.) Illumination at 0°C produces only a limited amount of chlorophyll. This limit is reached in a very short time. Continued illu-

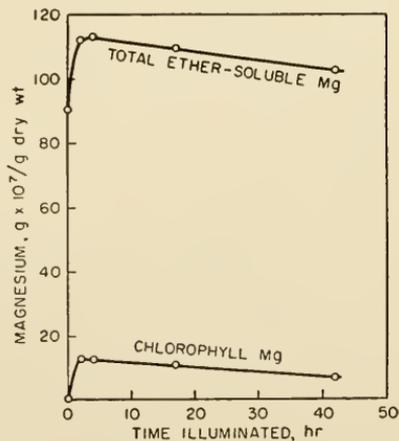


FIG. 7-8. Changes brought about in the content of total ether-soluble magnesium and chlorophyll magnesium by illumination of dark-grown barley leaves at 0°C. (Smith, 1949a.)

mination then causes destruction of the chlorophyll. These facts are pictured in Fig. 7-8 (Smith, 1949a).

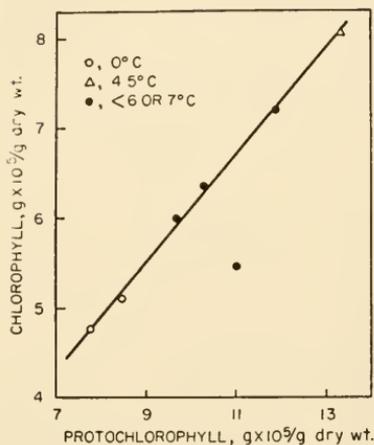


FIG. 7-9. The relation of the quantity of chlorophyll formed in dark-grown barley seedlings by illumination for 2 hr at low temperatures to the quantity of protochlorophyll initially present. Temperatures: 0°C; 4.5°C; below 6 or 7°C (ice bath). (Smith, 1948.)

When leaves containing various amounts of protochlorophyll were illuminated at low temperatures, they produced chlorophyll in direct proportion to the protochlorophyll they had contained. This is demonstrated in Fig. 7-9.

The transformation of protochlorophyll to chlorophyll a is quantitative. During illumination the protochlorophyll decreases and the chlorophyll a increases in such a way that the sum of the two remains constant even up to as high as 80 per cent conversion (see Table 7-4) (Koski *et al.*, 1951; Koski, 1950). These results bring cogent evidence that chlorophyll a is formed from protochlorophyll, molecule for molecule.

*Dependence of Chlorophyll Formation on Wave Length of Light.* There is general agreement among all investigators that the infrared radiation is ineffective in chlorophyll formation (Wiesner, 1877; Sayre, 1928; Guillemin, 1857; Reinke, 1893). The longest wave length effective is somewhat in doubt, but Sayre places this at 680  $m\mu$ , and Wiesner at less than 716  $m\mu$ . The

TABLE 7-4. THE CONSTANCY OF TOTAL PIGMENT DURING TRANSFORMATION OF PROTOCHLOROPHYLL TO CHLOROPHYLL A IN DARK-GROWN CORN LEAVES AFTER IRRADIATION FOR VARIOUS PERIODS OF TIME

Time, sec	Transformation, %	Protochlorophyll plus chlorophyll a, mg/g ( $\times 10^3$ )	Deviation from average, %
0	0.76	8.29	-3.7
10	12.0	7.89	-8.4
20	20.8	8.68	+0.8
40	34.7	8.64	+0.3
80	50.2	8.93	+3.7
160	69.8	8.55	-0.7
320	80.0	9.27	+7.7
		Av. 8.61	

long-wave-length limit is in the neighborhood of 700  $m\mu$ , perhaps little longer than 680  $m\mu$ .

All investigators agree that the visible spectrum is effective in the for-

mation of chlorophyll. Whether the relative activities of the different spectral regions have the order red > blue > green, as several investigators propose (Lubimenko, 1927; Monteverde and Lubimenko, 1911; Guerrini, 1941), or red > green > blue, as found by others (Sayre, 1928; Rudolph, 1934; Strott, 1938), is in doubt. The order may depend on the plant at the time of measurement, because great variation can occur in the effectiveness of the blue region (cf. Fig. 7-11).

It is uncertain how far the activity can extend into the ultraviolet (cf. Guillemin, 1857; Reinke, 1893; Wiesner, 1877; Stoklasa, 1911; Sayre, 1928; Weissenböck and Neubauer, 1940). Wiesner found greening up to about 397  $m\mu$ , whereas Sayre (1928), Stoklasa (1911), and Weissenböck and Neubauer (1940) place the limit at about 300  $m\mu$ .

Absolute limits cannot be given to the wave lengths of radiation active in chlorophyll formation, but the effective range is approximately from 680 to 300  $m\mu$ .

The action spectrum for the formation of chlorophyll was first measured quantitatively by Schmidt (1914), who illuminated etiolated corn leaves with various spectral regions isolated by means of calibrated colored filters. He assumed the wave length of irradiation to be the middle of the filter's transmission band. He calculated the incident energy from the light intensity and the time necessary to develop the first spectroscopically detectable chlorophyll. The reciprocal of this energy he considered as proportional to the effectiveness of the different wave lengths. Schmidt's results follow:

Wave length, $m\mu$ . . . . .	677.5	640.0	620.0	592.5	567.5	527.5	505.0	477.5	450.0	432.5
Relative effectiveness . . . . .	2.95	123.1	17.25	67.5	116.5	5.92	1.2	3.22	96.15	13.40

Schmidt found three peaks of effectiveness at wave lengths 640, 567, and 450  $m\mu$ . The relative efficiencies of the radiation were in the order given.

Frank (1946) measured the effectiveness of 16 regions of the spectrum for the production of chlorophyll in oat seedlings. She used combinations of light filters to isolate the various spectral regions, and to each filter combination she assigned a dominant wave length. She employed an illumination period of 5 hr with an energy flux sufficient to form a definite quantity of chlorophyll. The chlorophyll formed was extracted and was measured spectroscopically. The effectiveness was then calculated as the reciprocal of the relative number of quanta required in different parts of the spectrum to produce the same quantity of chlorophyll. The effectiveness curve obtained is given in Fig. 7-10 along with the absorption curve of protochlorophyll in ether (cf. Smith, 1948). Peaks of effectiveness, in the order of their efficiency, were found at 445, 645, 575, and 545  $m\mu$ .

Koski *et al.* (1951) measured the action spectra for the transformation of protochlorophyll to chlorophyll a in dark-grown normal and albino corn leaves. The leaves were illuminated with various wave lengths of

monochromatic light—band width  $5\text{ m}\mu$ —obtained by means of a large-aperture grating monochromator. The light source was a high-pressure mercury lamp. Illumination periods of 20–360 sec were used. The light intensity was measured with a thermopile, and the total energy (quanta) incident on the leaves calculated. The extent of the pigment transformation was determined from spectroscopic measurements. The reciprocal

of the number of quanta necessary to produce 20 per cent transformation was used as a measure of the effectiveness at each wave length. The curves of effectiveness vs. wave length are given in Fig. 7-11 and compared with the absorption of protochlorophyll in methanol.

For the normal sib the maxima of effectiveness are at 650, 445, 593, and 550  $\text{m}\mu$ , and the relative efficiencies are in this order. However, for the albino white seedling-3, the order is 445, 650, 593, and 550  $\text{m}\mu$ . The ratio of effectiveness for the blue and red peaks is 0.66 for the normal and 1.89 for the albino. The lower effectiveness of blue light for the normal sibs may be ascribed to the screening effect of the carotenoid pigments, which are abundant in the normal seedlings but lacking in the albino. It is difficult to understand Frank's results for the high effectiveness of blue light relative to red in normal oat seedlings, because it is evident from Fig. 7-1

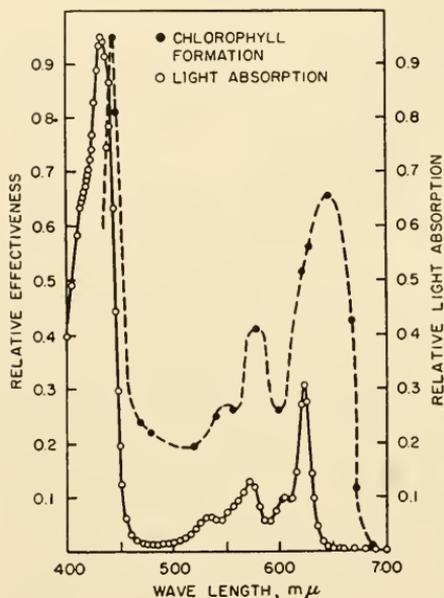


FIG. 7-10. The relative-effectiveness curve for chlorophyll formation (Frank, 1946) compared with the relative-light-absorption curve of protochlorophyll dissolved in ether. The relative-light-absorption curve of protochlorophyll has been adjusted so that the height of the chief absorption band equals the height of the corresponding band in the relative-effectiveness curve (Smith, 1948).

that her seedlings contained an abundance of carotenoid pigments, which absorb strongly in the blue. Frank attributed this anomaly to a particular geometrical arrangement of the pigments in the leaf. [For a discussion of this point see Frank (1946), Koski *et al.* (1951), Aronoff (1950).]

A comparison of the action-spectrum curves with the absorption-spectrum curve (Fig. 7-11) shows that the shapes of the curves differ but that the positions of the maxima are in agreement when allowance is made for the spectral shift between solvent extracts and holochrome. On a frequency basis the shift for methanol is  $151.8 \times 10^{11}$  frequency units per second and for ether,  $199.3 \times 10^{11}$  (Koski *et al.*, 1951).

When part of the protochlorophyll was transformed to chlorophyll and the leaves were illuminated with light absorbed predominantly by chlorophyll (wave length  $680\text{ m}\mu$ ), the light absorbed by chlorophyll was ineffective in the transformation (*ibid.*).

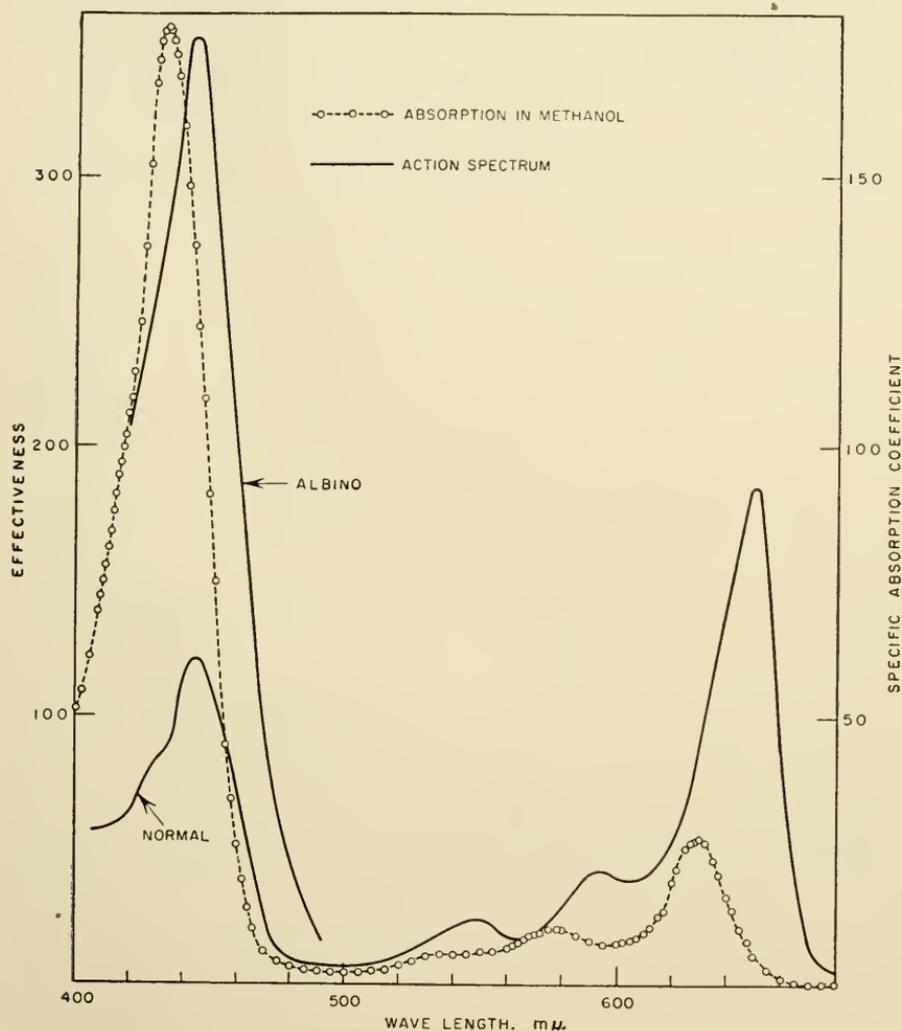


FIG. 7-11. Action spectra for the transformation of protochlorophyll to chlorophyll in dark-grown normal and albino corn seedlings (solid line), compared with the absorption spectrum of protochlorophyll in methanol (broken line). The effectiveness values for the normal and albino seedlings are equal in the long-wave-length band of the action spectra. (Koski *et al.*, 1951.)

These results leave little room for doubt that protochlorophyll holochrome is the active light absorber for its own transformation.

After considerable amounts of chlorophyll have been formed, it is hardly feasible to obtain a significant action spectrum for the partici-

pation of a minor pigment, such as protochlorophyll, in the chlorophyll-forming process, even though such a pigment is known to be present in normal green leaves (Smith and Koski, 1947-1948). This arises from several causes: the effectiveness curve obtained would be seriously distorted by the inner-filter action of chlorophyll; the additional chlorophyll formed would be quantitatively insignificant; and the photosynthetic action spectrum of chlorophyll itself would play a dominating role.

*Rate of Formation of Chlorophyll.* There is no question but that the first trace of chlorophyll appears in etiolated leaves after only a few seconds' exposure to light of moderate intensity. Monteverde (1893-1894) placed this time at 5 sec in diffuse daylight. Liro (1908) found that chlorophyll was formed in spectroscopically determinable amounts in 5-10 sec by irradiation with diffuse daylight. In some plants 30 sec was sufficient to transform all the "leukophyll" (protochlorophyll). The time depended on the thickness of the plant part being illuminated. Inman (1935) exposed etiolated corn leaves to radiation from a 100-w Mazda lamp at 8-in. distance and detected chlorophyll spectroscopically after 10 sec and a typical chlorophyll spectrum after 150 min. The present authors (Fig. 7-14; cf. Koski, 1949) found that in corn leaves 10 sec of exposure converted 10.3 per cent of the protochlorophyll to chlorophyll at 30 ft-c intensity, 43.8 per cent at 120 ft-c, and 59.3 per cent at 240 ft-c. The transformation of protochlorophyll to chlorophyll in etiolated corn leaves brought about by irradiation with weak monochromatic light, wave length 650 m $\mu$ , conforms to the second-order reaction-rate law (cf. Table 7-4).

From these results it is clear that, at moderate light intensities, chlorophyll can be detected after 5 sec or less of irradiation (cf. Liro, 1908, pp. 28-29). The reaction is a photochemical process in which the photochemically active component is used up.

The rate of accumulation of chlorophyll is a slow reaction as compared with the transformation of protochlorophyll (cf. Figs. 7-12 and 13; see also Liro, 1908, pp. 90-92). After the initial transformation of protochlorophyll there is a lag period in which little additional chlorophyll is formed; then chlorophyll increases at an accelerated rate suggestive of an autocatalytic process in which the chlorophyll accumulated contributes to its own formation. This probably happens through the accelerated photosynthesis of substances contributory to chlorophyll production. Once this autocatalytic phase is over, the process slows down until a steady state is reached. The steady state is considered by some to be a balance between the production and destruction of chlorophyll (Zavallishina, 1951).

*Dependence of Chlorophyll Formation on Light Intensity.* The minimum intensities of light which bring about chlorophyll formation are very low. Tessier (1783) observed that the light of the moon was sufficient to green

leaves perceptibly, and Issatschenko (1907) claimed that chlorophyll is formed in bacterial light.

There is evidently a threshold light intensity below which protochlorophyll is not transformed to chlorophyll. Lubimenko (1928) found that etiolated barley plants illuminated for 10 hr with relative light intensities of 100, 11.1, and 6.25 formed relative quantities of chlorophyll of 100, 21, and 0. These data demonstrate that a threshold intensity exists for chlorophyll formation.

At relatively low light intensities the rate of formation of chlorophyll is directly proportional to the light intensity (Wiesner, 1877; Schmidt,

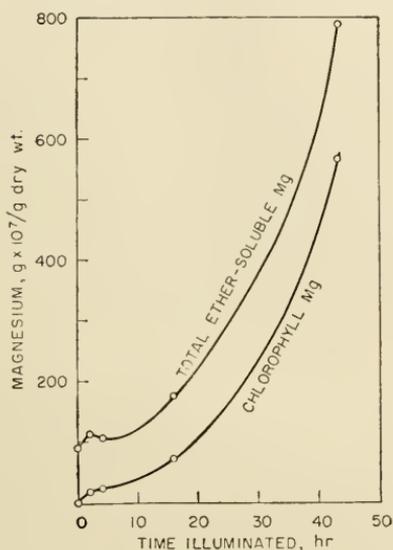


FIG. 7-12. Changes brought about in the content of total ether-soluble magnesium and chlorophyll magnesium by illumination of dark-grown barley leaves at 7°C. (Smith, 1949a.)

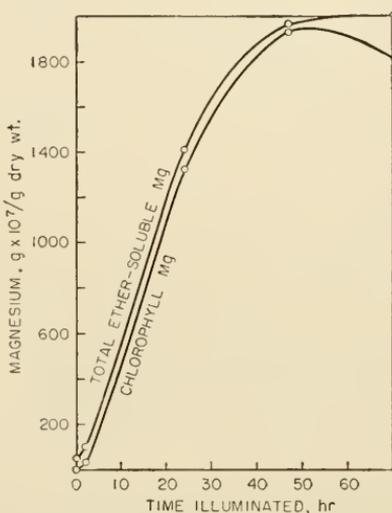


FIG. 7-13. Changes brought about in the content of total ether-soluble magnesium and chlorophyll magnesium by illumination of dark-grown barley leaves at 19°C. (Smith, 1949a.)

1914; Greilach, 1904; Liro, 1908; Koski and Smith, 1948-1949; Koski, 1949).

This was convincingly demonstrated by Liro (1908), who investigated the transformation of protochlorophyll ("leukophyll") to chlorophyll in two ways: by measuring the time necessary to form the first spectroscopically detectable trace of chlorophyll at different distances from a given light source, and by measuring the dilution necessary to cause the disappearance of the chlorophyll band produced by a constant time of illumination with a given weak light source at different distances. From Liro's results it is evident that the rate of formation of the first trace of chlorophyll is proportional to the light intensity as measured by the inverse-square law.

Schmidt (1914) confirmed these results and demonstrated that the law applied to different wave lengths.

The present authors (Koski and Smith, 1948-1949; Koski, 1949) followed the time course of the transformation at different light intensities (cf. Fig. 7-14). At the lowest intensity the rate followed a second-order law, but the rate law changed with the intensity; this indicated that the reaction was complicated by certain factors, which have not yet been determined. Nevertheless in the early stages of the reaction the rate

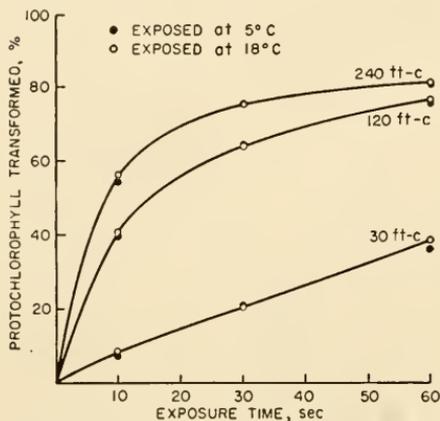


FIG. 7-14. The time course of the transformation of protochlorophyll to chlorophyll at different light intensities and at temperatures of 5° and 18°C. (Koski, 1949.)

was approximately proportional to the intensity. Intense light inhibits the formation of chlorophyll. Scharfnagel (1931; cf. Noack, 1934) observed that by illuminating an etiolated corn leaf with very intense light he could suspend the transformation of protochlorophyll to chlorophyll. The suspension was only temporary because transfer of the leaf to lower light intensities permitted the transformation to take place.

Lubimenko (1928; cf. Monteverde and Lubimenko, 1912) has shown that exposure of etiolated cotyledons to direct sunlight decreases the rate of chlorophyll accumulation by subsequent illumination with weaker light. Three lots of pumpkin cotyledons were exposed to direct sunlight for 0, 5, and 10 min and then placed in diffuse daylight for 7 hr. The relative quantities of chlorophyll produced were, respectively, 100, 72, and 62. Lubimenko concludes that light influences not only the transformation of "chlorophyllogène" to chlorophyll but also other reactions allied with the production of pigments; this is why such complex relations are sometimes observed between light intensity and the accumulation of chlorophyll.

Sargent (1940) observed that *Chlorella* cells grown at high light intensities contain less chlorophyll than those grown at weaker intensities; however, because of the greater growth in full sunlight, they produce a greater total amount of chlorophyll at the higher intensities. He found a ratio of 0.51 for the chlorophyll content of cells grown at full sunlight intensity versus that at one-seventh this intensity, but a ratio of 2.5 for the rates of production of chlorophyll at the two intensities.

Myers (1946) has also determined the rate of production of chlorophyll in *C. pyrenoidosa* as a function of light intensity. The rate increases almost linearly with increase in light intensity at low intensities, reaches a

maximum at about 55 ft-c, and thereafter slowly decreases as the light intensity increases. The results derived from Myers's data are shown in Fig. 7-15.

The accumulation of chlorophyll is a local effect. Only the illuminated portion of an etiolated leaf becomes green. According to Strain (1949): "If this partially green seedling is now returned to the dark for several days, the green portion fades very much. Upon exposure of the entire seedling to light, chlorophyll forms faster in the previously unexposed portion than in the exposed and faded portion."

*Dependence of Chlorophyll Formation on Intermittent Light.* It has been known since the time of Mikosch and Stöhr (1880) that the same quantity of irradiation produces more chlorophyll when administered intermittently than when administered continuously. These workers found that barley plants at 20°C accumulated the same quantity of chlorophyll with 4.5 hr of illumination, part of which was intermittent, as with 6.5 hr of continuous illumination (cf. Biebel, 1942).

Experiments of Mikosch and Stöhr (1880) also indicate that the transformation of protochlorophyll to chlorophyll is more efficient in intermittent than in continuous illumination. Barley and oat seedlings produced more chlorophyll during 5 min of intermittent lighting (1-sec periods) than during 2.5 min of continuous lighting. Also cress seedlings produced a detectable quantity of chlorophyll in 12.5 min of intermittent illumination but none in 6.25 min of continuous illumination. This type of experiment will bear repetition.

The authors have determined the effect of intermittent illumination on the formation of chlorophylls a and b. Their results are graphed in Fig. 7-16. In this experiment the plants were exposed 5 min out of every hour. It may be seen that less of each chlorophyll is accumulated during the same period of elapsed time for intermittent than for continuous illumination, but that, on the basis of total light energy, the amount of chlorophyll a accumulated is more than double for the intermittent illumination and the amount of chlorophyll b is somewhat less than double. Because the accumulation of chlorophyll is greater for a given amount of radiation administered intermittently rather than continuously, the formation of chlorophyll is undoubtedly a combination of slow thermochemical and rapid photochemical processes. The product of the thermo-

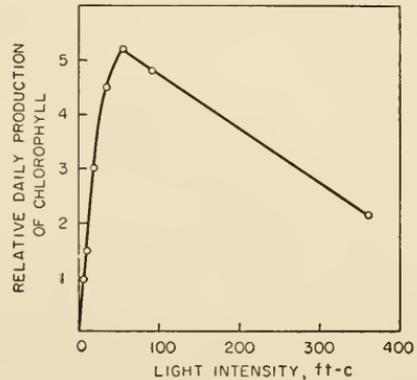


FIG. 7-15. Relative amounts of chlorophyll produced daily by *Chlorella pyrenoidosa* at different light intensities. [Derived from Myers's data (Myers, 1946).]

chemical process is transformed to chlorophyll by photochemical action.

Since the immediate precursor of chlorophyll is protochlorophyll, it is of interest to determine how protochlorophyll behaves in etiolated seedlings under intermittent illumination.

The amount of chlorophyll formed from the protochlorophyll initially contained in dark-grown leaves is too small to impart a green color to the leaves. In order for protochlorophyll to be the precursor of chlorophyll in the over-all process of greening, it must be continually formed and transformed. Protochlorophyll behaves in this manner (Scharfnagel,

1931; Lubimenko and Hubbenet, 1932).

The rate of re-formation of protochlorophyll has been measured by Scharfnagel (1931), by Rudolph (1934), and by the authors. Scharfnagel irradiated etiolated corn seedlings for 10 min to transform the protochlorophyll to chlorophyll and then placed the plants in darkness. After 1, 2, and 3 hr he extracted the seedlings and found no re-formation of protochlorophyll. After 10 hr protochlorophyll was barely perceptible, and after 20 hr it was present in significant amounts. By alternating periods of light and darkness he was able to demonstrate the disappearance of protochlorophyll in the light and appearance in the dark.

Experiments in the authors' laboratory have also demonstrated the

rate of regeneration of protochlorophyll in the dark for normal and albino corn seedlings. The results are presented in Table 7-5.

The accumulation of protochlorophyll reaches a limiting value in seedlings that have been grown in the dark and also in dark-grown seedlings that have been briefly illuminated and returned to the dark (Lubimenko and Hubbenet, 1932).

*Polarized Light.* Tessier (1783; see Guillemin, 1857) found that "la matière verte" is formed in red, yellow, blue, and violet light that has been polarized by means of a Nicol prism.

*Photochemical Formation of Additional Organic Magnesium Compounds.* When etiolated barley leaves are illuminated at 0°C, their protochloro-

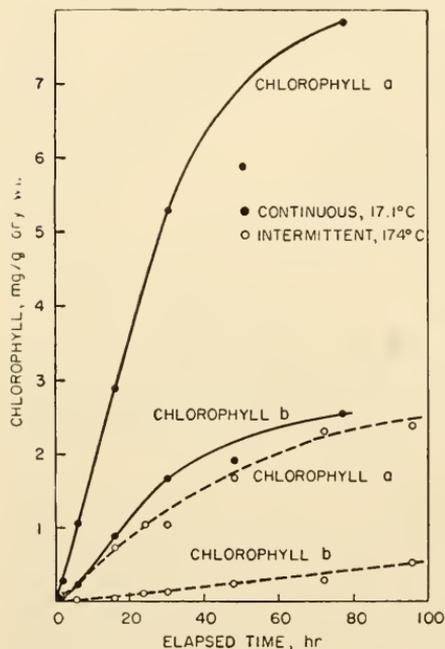


FIG. 7-16. The accumulation of chlorophylls a and b by barley seedlings in continuous and intermittent illumination.

phyll is transformed to chlorophyll. This involves no change in total ether-soluble magnesium. But inasmuch as illumination more than doubles the quantity of magnesium extractable with ether, it must be concluded that new organic magnesium compounds are formed by photochemical action (cf. Fig. 7-8). It is not known that these compounds are precursors of chlorophyll, but their nature suggests this possibility (Smith, 1949a).

TABLE 7-5. REGENERATION OF PROTOCHLOROPHYLL IN DARK-GROWN CORN SEEDLINGS

Leaf type	Protochlorophyll content, $\mu\text{g/g}$ fresh weight of leaves			
	Initial	3 min light + 0 hr dark	3 min light + 5 hr dark	3 min light + 74 hr dark
Normal.....	2.48	0.464	1.113	2.28
Albino.....	3.21	0.518	0.805	1.94

#### 4. EFFECT OF TEMPERATURE ON CHLOROPHYLL FORMATION AND ACCUMULATION

A systematic study by Sachs (1865) showed that, within limits, the rate of greening increased with the temperature, that there was a threshold temperature below which each species of plant would not green, and that this threshold differed with the species.

TABLE 7-6. EFFECT OF TEMPERATURE ON THE ACCUMULATION OF CHLOROPHYLL IN *Triticum ferrugineum* AFTER VARIOUS LENGTHS OF ILLUMINATION (Lubimenko, 1928.)

Temperature, $^{\circ}\text{C}$	Relative quantities of chlorophyll					
	2 hr	8 hr	16 hr	24 hr	48 hr	72 hr
0	1	0.9	0.8	0.8	0.8	0.8
4	1	1.1	....	3.3	9	15
11	1	3.4	7.5	12	22	35
20	1	...	10	16	20	32

Lubimenko (1928) followed the rate of chlorophyll formation at different temperatures. His results for *Triticum ferrugineum* are given in Table 7-6. After 2 hr of illumination a small quantity of chlorophyll is formed at all temperatures. At  $0^{\circ}\text{C}$  the quantity of chlorophyll is not increased by further illumination; at  $4^{\circ}\text{C}$  it remains about constant for 8 hr and then increases at an accelerated rate; and at  $11^{\circ}$  and  $20^{\circ}\text{C}$  it

increases at considerably greater rates and with a shorter induction period than at 4°C.

Experiments carried out by Smith (1949a) gave similar results, shown in Figs. 7-8, 12, and 13. These curves, besides showing the course of chlorophyll accumulation, display the behavior of the total ether-soluble magnesium fraction at different temperatures. The results may be interpreted in the following manner: At low temperature, 0°C, the limited amount of chlorophyll precursor (protochlorophyll) present in the leaves is transformed photochemically to chlorophyll, but no further precursor

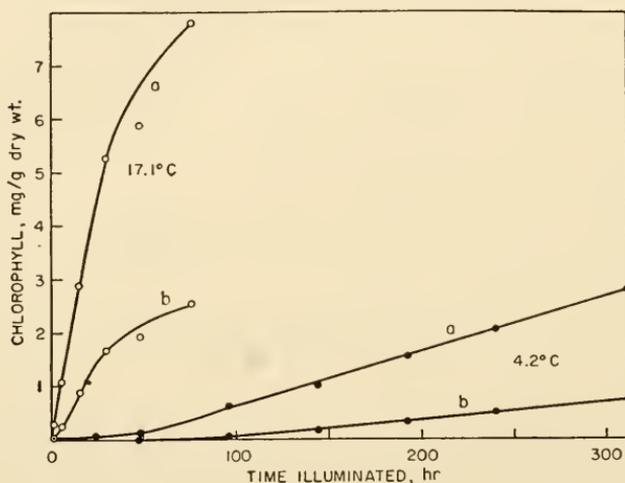


FIG. 7-17. The effect of temperature on the accumulation of chlorophylls a and b in dark-grown barley seedlings.

is formed; at intermediate temperatures, e.g., 7°C, after the initial precursor has been transformed, time is required to form additional precursor. Following this induction period, precursor formation, and consequently chlorophyll accumulation, is accelerated. This acceleration may possibly result from accelerated photosynthesis. At favorable temperatures, e.g., 19°C, the induction period is shortened so as to be almost indiscernible, and the acceleration of chlorophyll accumulation is compensated by a deceleration, so that the increase of chlorophyll is almost constant with time until the steady-state condition is nearly reached. The photochemical formation of other organic magnesium compounds at 0°C and their persistence during the whole course of chlorophyll accumulation at other temperatures are clearly evident.

The effect of temperature is a local phenomenon. Two parts of an etiolated leaf illuminated at two temperatures, one below and the other above the threshold temperature for greening, show a distinct line of demarcation between them. The part kept at low temperature remains yellow; the part kept at the higher temperature becomes green (*ibid.*).

Temperature affects the formation of both chlorophylls a and b and to approximately the same extent. In Fig. 7-17 are shown the rates of formation of chlorophylls a and b in barley seedlings at 4.2° and 17.1°C. Although the absolute rates of formation are greatly affected by temperature, the relative rates of formation of chlorophylls a and b remain nearly constant over a considerable range of chlorophyll concentrations and are approximately the same for different temperatures, as shown in Fig. 7-18 (see also Seybold and Egle, 1938; Blaauw-Jansen *et al.*, 1950).

There is an optimum temperature for the accumulation of chlorophyll, as Lubimenko and Hubbenet (1932) have clearly shown (Fig. 7-19).

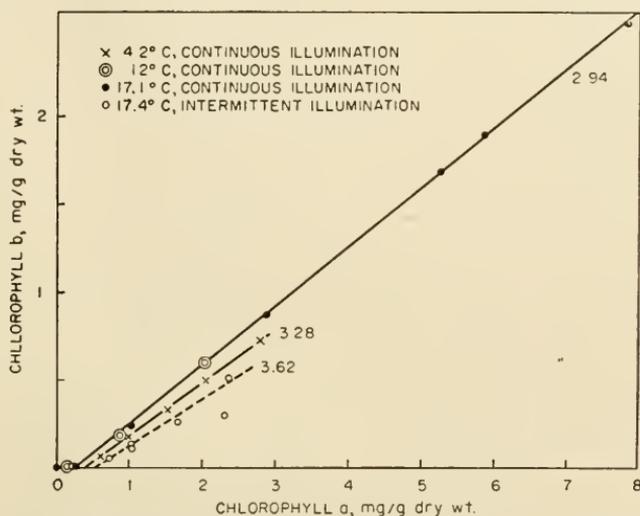


FIG. 7-18. The relation between the accumulation of chlorophylls a and b at different temperatures under continuous and intermittent illumination of dark-grown barley seedlings. The numbers adjacent to the lines designate their slopes. (Smith, 1949b.)

From the graph it is seen that the optimum temperature is about 27°C for wheat seedlings (*Triticum ferrugineum*). The range of temperature for this plant which permits chlorophyll accumulation is from 3° to 48°C. Plants heated at 47°C for 48 and 72 hr died. Strain (1938, p. 124) found that immersion of barley seedlings in water at 50°C for 30 sec inhibited chlorophyll formation. Different plants have different optimal temperatures for chlorophyll formation (Wiesner, 1877).

Inman (1940) observed that certain algae that grow in hot springs at temperatures of 37°–72°C retain their chlorophyll. The ratio of chlorophyll a to chlorophyll b is greater than is commonly found in most plants.

In contrast to the great dependence of the accumulation of chlorophyll on temperature, the transformation of protochlorophyll to chlorophyll is quite independent of temperature (Lubimenko, 1928). Liro observed that chlorophyll was formed in illuminated etiolated seedlings at temperatures as low as -15°C. Scharfnagel (1931) obtained chlorophyll for-

mation in etiolated corn seedlings illuminated at  $-6^{\circ}\text{C}$ . But in none of these experiments was the rate of formation determined at different temperatures and at different light intensities. This has been done by Koski and Smith (1948-1949; cf. Koski, 1949). The results (Fig. 7-14) show that the rates are nearly identical at  $5^{\circ}$  and  $18^{\circ}\text{C}$  and at light intensities of 30, 120, and 250 ft-c.

Etiolated leaves that have had their original protochlorophyll transformed to chlorophyll re-form protochlorophyll when returned to the

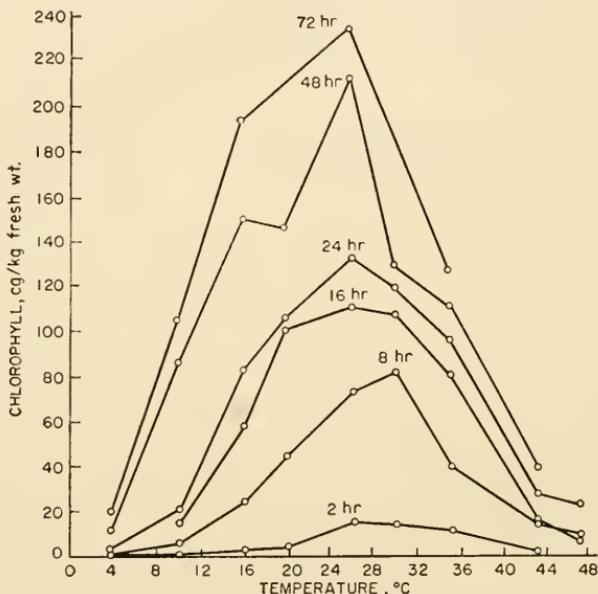


FIG. 7-19. Curves showing the total production of chlorophyll at different temperatures after 2, 8, 16, 24, 48, and 72 hr of exposure of wheat (*Triticum ferrugineum*) seedlings to light. (Lubimenko and Hubbenet, 1932.)

dark at  $19^{\circ}$ , but not at  $0^{\circ}\text{C}$  (Smith and Koski, 1947-1948). They do not transform protochlorophyll to chlorophyll after they have been heated to a temperature of  $90^{\circ}$ - $95^{\circ}\text{C}$  (Koski and Smith, 1948).

##### 5. INFLUENCE OF THE AMBIENT ATMOSPHERE ON THE FORMATION AND ACCUMULATION OF CHLOROPHYLL

*Oxygen.* Certainly in the higher plants oxygen is necessary for greening. Boehm (1865) determined that, in atmospheres of pure nitrogen, hydrogen, or carbon dioxide, etiolated plants did not green. They greened only when oxygen was present. Correns (1892), Friedel (1902), and Trebitz (1905) have all demonstrated that greening is regulated by the partial pressure of the oxygen surrounding the plant and that there is a threshold oxygen pressure below which they will not green. Individuals of the same species vary in this regard (Correns, 1892; Liro, 1908).

Of 16 species examined by Trebitz (1905), the cereals *Hordeum distichum*, *Triticum sativum*, and *Avena sativa* required the lowest air pressure, 10 mm Hg, and *Lepidium sativum*, the highest, 60 mm Hg.

Experiments of Friedel (1904) indicated that the quantity of oxygen rather than the pressure was the determining factor in producing greening, but Liro (1908) concluded from his experiments that not only the absolute amount of oxygen but also its pressure affects the rate of greening.

The action of oxygen is local, because etiolated leaves half submerged in water green only in the section freely exposed to air (Trebitz, 1905).

Oxygen is involved in the thermochemical and not the photochemical part of the greening process. Liro (1908, pp. 103 ff.) demonstrated this by alternately placing etiolated leaves in the light at low air pressures and in the dark at normal air pressures. These leaves became green. In a comparable experiment he illuminated etiolated leaves continuously at low air pressures. Those leaves scarcely greened, if they greened at all. During the sojourn in the dark at normal air pressure, the leaves form chlorophyll precursor, which they convert to chlorophyll when brought into the light. But at the lower air pressures they are unable to form the precursor, and consequently they do not green.

Although oxygen is necessary for the greening of etiolated leaves, it is not necessary for the transformation of protochlorophyll to chlorophyll. Liro (1908) demonstrated that traces of chlorophyll are formed in etiolated leaves illuminated in an atmosphere of hydrogen from which all the oxygen had been removed by treatment with pyrogallol, and Smith (1950-1951) obtained an 80 per cent conversion of protochlorophyll to chlorophyll a in a hydrogen atmosphere containing less than  $10^{-7}$  part oxygen. But Scharfnagel (1931) got only the barest trace of transformation in an atmosphere of nitrogen rigorously freed of oxygen. This finding needs to be reexamined.

Frenkel and Rieger (1951) have evidence that the pigments of *Porphyridium cruentum* increase under anaerobic conditions. In lower photosynthetic forms, e.g., Athiorhodoceae, the characteristic chlorophylls are also formed in the absence of oxygen (cf. Van Niel, 1944).

*Carbon Dioxide.* Chlorophyll does not accumulate in etiolated plants illuminated in an atmosphere of pure carbon dioxide (Boehm, 1865; Correns, 1892). Trebitz (1905) found that at 1 per cent carbon dioxide neither an acceleration nor an inhibition of greening was apparent, but that retardation was evident at 2-5 per cent. The minimum carbon dioxide concentration that stops greening varies with the plant species: for *Lupinus albus* it lies between 7 and 17 per cent, and for the cereals *Hordeum distichum*, *Avena sativa*, and *Triticum sativum* it lies between 50 and 70 per cent.

Carbon dioxide is not essential for the formation of chlorophyll, as Rudolph (1934) and Rombeck (1943) have shown. In some cases, how-

ever, the amount of chlorophyll accumulated appeared to be less in a carbon dioxide-free atmosphere than under normal conditions (*ibid.*).

*Other Gases.* Ozone was found by Boehm (1865) to be detrimental to chlorophyll accumulation.

Carbon monoxide decreases the rate of chlorophyll formation (Yocum, 1946).

Sulfur dioxide and vapors of ether, fuming nitric acid, and ammonium thiocyanate in concentrations insufficient to kill the leaf have little or no effect on the protochlorophyll-chlorophyll transformation (Scharfnagel, 1931).

## 6. INFLUENCE OF NUTRITION ON THE FORMATION AND ACCUMULATION OF CHLOROPHYLL

Are the constituent elements of chlorophyll derived directly from the inorganic components of the nutrient medium?

A direct photosynthetic utilization of carbon dioxide for chlorophyll formation is not indicated because of the following facts: Chlorophyll is formed in plants illuminated in air free of carbon dioxide (Rombeck, 1943), and chlorophyll extracted from plants that have photosynthesized labeled carbon dioxide does not contain labeled carbon in excess of other substances formed concurrently (Ruben *et al.*, 1939; Clendenning, 1950). Chlorophyll also is found in organisms that do not utilize carbon dioxide photosynthetically (Davis, 1948).

The hydrogen contained in chlorophyll is undoubtedly derived from organic intermediates, not directly from water. No labeled hydrogen (tritium) was found in the chlorophyll obtained from *Chlorella pyrenoidosa* cells that had photosynthesized for 3 hr in tritium oxide (Norris *et al.*, 1942). Also the proportion of labeled hydrogen (deuterium) was the same in the porphyrin and phytol fractions of chlorophyll produced during photosynthesis in deuterium oxide by *Chlorella* cells (Calvin and Aronoff, 1948). This suggests that the hydrogen in chlorophyll comes from the metabolism of organic compounds rather than directly from water.

The case of nitrogen is not so clear, because the chlorophyll content of organisms grown in media of different nitrogen concentrations is proportional to the nitrogen concentration. Fleischer (1935) demonstrated that cells of *Chlorella* Cornell No. 11 (cf. Mandels, 1943) could be grown with very small chlorophyll content when the nitrogen content of the nutrient medium was very low, and that the chlorophyll content of the cells could be increased by increasing the nitrogenous nutrients. When the nitrogen was changed from 10 to 80 ppm, the chlorophyll content of the cells was changed from  $1 \times 10^{-5}$  to  $13 \times 10^{-5}$  per 10 mm<sup>3</sup> of packed cells. Van Hille (1938) cultivated *C. pyrenoidosa* cells in two nutrient solutions containing potassium nitrate in concentrations in the ratio of 4 to 1. The

chlorophyll produced per unit volume of these culture solutions was in the ratio of 4.1 to 1. Thus there appears to be a direct proportionality between the nitrogen in the medium and the chlorophyll. These results do not necessarily mean, however, that the nitrogen was used directly for the formation of chlorophyll, because in other experiments organic nitrogen compounds have been shown to serve as sources of nitrogen (cf. Ludwig, 1938; Aronoff, 1950; Salomon *et al.*, 1950). In fact, there appears to be a correlation between protein metabolism and the chlorophyll content of the leaf (Rombeck, 1943).

The fact that magnesium is one of the constituent elements of chlorophyll makes its presence in the nutrient medium obligatory for the continued formation of chlorophyll. Mameli (1915) states that plants of the same species cultivated in solutions containing various quantities of magnesium develop foliage with a chlorophyll content directly proportional to the magnesium administered. Fleischer (1935) observed that *Chlorella* Cornell No. 11 became colorless when it was deprived of magnesium, and by variation of the magnesium content of the culture medium in which it was grown, it could be brought to various degrees of greenness. When the magnesium content was increased from 0.02 to 2.0 ppm, the chlorophyll content was increased from  $10^{-5}$  to  $12 \times 10^{-5}$  g per cubic millimeter of cells. Van Hille (1938) cultivated *C. pyrenoidosa* cells in nutrient media of various magnesium concentrations. The growth of cells was dependent on the magnesium content of the media, but the chlorophyll content of the cells was about the same in all the cultures. The total chlorophyll produced per unit volume of culture medium increased almost linearly with the magnesium up to about  $4 \times 10^{-7}$  g of magnesium per cubic centimeter. Above this there was no further increase. Bukatsch (1941-1942) found that corn, wheat, and barley plants cultivated in nutrient solutions increased in chlorophyll when the magnesium in the nutrient solution was increased from zero to "normal," but further increase in the magnesium had little or no effect on the chlorophyll. Rissmann (1930) obtained a difference in the reaction of wheat and corn seedlings to magnesium added to their nutrient solutions. This dissimilarity he attributed to the unequal magnesium content of the seeds.

Rissmann (1930) and Smith (1947) have shown that dark-grown seedlings contain organic magnesium compounds. Of the total magnesium thus combined in barley seedlings, from about 23 to 46 per cent has been found to be attributable to protochlorophyll (Smith, 1949a). When etiolated leaves are illuminated, inorganic magnesium is brought into organic combination (cf. Fig. 7-8).

Many elements that are not constituents of extracted chlorophyll or protochlorophyll affect the formation of chlorophyll. A discussion of their hypothetical involvement in pigment formation is beyond the scope

of this review. As an introduction to this phase of the subject, the reader is referred to Bear and Coleman (1949), Pirson (1948), Pirschle (1938, 1939), Mandels (1943), and Aronoff (1950).

*Organic Nutrition.* Carbohydrates are essential to the accumulation of chlorophyll, according to Palladin (1918). Some etiolated leaves, e.g., wheat, are well supplied with carbohydrate, whereas others, e.g., bean and lupine, are not. When floated on water and illuminated, leaves of the former type become green, whereas those of the latter type remain yellow. If, however, carbohydrate-poor leaves are floated on sugar solutions and illuminated, they too become green. The concentration of the sugar solution plays a role: too concentrated solutions are inhibitory to greening (see later). Palladin (1897), by the floating-leaf technique, found several substances to favor chlorophyll formation: sucrose, raffinose, glucose, fructose, maltose, glycerin, galactose, lactose, and dextrin; other substances were found to be without effect: inulin and tyrosine; and still other substances completely inhibited the formation of chlorophyll: mannitol, dulcitol, asparagine, urea, and alcohol.

Zaitseva (1940) found that removal of the endosperm from germinated wheat seedlings greatly reduced the amount of chlorophyll formed in the illuminated seedling unless sucrose was administered to it. (For the effect of nutrients on the formation of chlorophyll in the dark, see Sect. 7.)

*Inhibition of Chlorophyll Formation.* Inhibitors to the formation of chlorophyll have been studied in order to get information concerning the mechanism of the process. The specific effects of several inorganic substances have been determined.

Boehm (1859) found that etiolated plants, when illuminated in an atmosphere containing ozone, became bleached. Yocum (1946) observed that 0.001 *M* cyanide stopped chlorophyll accumulation but did not inhibit the transformation of protochlorophyll to chlorophyll in dark-grown bean seedlings. Carbon monoxide-oxygen mixtures (95 per cent CO, 5 per cent O<sub>2</sub>) reduced chlorophyll formation to about 50 per cent of the values obtained in a nitrogen-oxygen mixture of the same oxygen concentration when etiolated bean leaves were illuminated with red light. But when they were illuminated with blue light, the carbon monoxide inhibition was completely reversed. Respiration of the leaves followed much the same pattern. From these experiments Yocum concluded that "Since protochlorophyll formation is dependent on the functioning of a respiration system which shows a reversible cyanide and a light reversible CO inhibition it is suggested that cytochrome oxidase is essential for protochlorophyll synthesis." According to Euler (1949), seeds soaked in cobalt nitrate solutions, 0.004 *M*, failed to produce seedlings that greened. He attributed the effect to inactivation of the phosphatases in the mitochondria.

The action of organic vapors has been studied by various workers.

Trebitz (1905) found that alcohol and ether vapors that were not injurious to the plant did not inhibit chlorophyll formation except in one plant, *Pisum sativum*. Liro (1908) observed that seedlings of barley, oats, and wheat, after a half-hour treatment with higher concentrations of ether and chloroform vapor, turned completely white when illuminated. Neither protochlorophyll nor chlorophyll could be detected in alcoholic extracts of these seedlings. Scharfnagel (1931) reported that narcosis brought about with various amounts of ether produced only a negligible inhibition of the protochlorophyll-chlorophyll transformation in corn seedlings. Brebion (1950) related the inhibiting action of various substances to their thermodynamic activities, i.e., for vapors, the fraction of their full vapor pressure which causes inhibition. For the inhibiting activities on chlorophyll formation in wheat seedlings, he gives the following values: ether, 0.35; acetone, 0.2; benzene 0.2; and phenol, 0.06. Brebion has also observed that substances that inhibit chlorophyll formation at one concentration may stimulate it at a lower concentration. He has given the ratios of inhibitory action to stimulatory action for various compounds. Strain [cf. Spoehr *et al.* (1938-1939)] has reported the inhibitory action of hydroquinone on the greening of barley seedlings.

Treatment of barley seeds with streptomycin previous to germination inhibited chlorophyll production in the seedlings (Euler, 1947, 1950). Euler ascribed the action to a deleterious effect on chloroplast formation (Euler and Heller, 1949) and to an obstruction of porphyrin synthesis (Euler, 1950). Cells of *Euglena gracilis* are bleached in the light when treated with streptomycin (Provasoli *et al.*, 1948). The bleaching is proportional to the extent of the treatment. Microscopic examination revealed drastic reduction in the number of chloroplasts. Completely bleached cells never recovered their ability to form chlorophyll. Bogorad (1950b) observed that seeds of *Pinus jeffreyi* when germinated in the dark on agar containing 0.2 per cent streptomycin produced seedlings with colorless cotyledons in contrast to the green cotyledons produced under normal conditions. When the colorless seedlings were exposed to light, they became green.

Another compound that, in very low concentrations, adversely affects the formation of chlorophyll is 3-( $\alpha$ -imino-ethyl)-5-methyl-tetronic acid (Alamercery *et al.*, 1951).

Sugars may act as inhibitors of chlorophyll production in certain organisms. Kufferath (1913) obtained chlorotic cells of *Chlorella luteo-viridis* when they were illuminated and grown on an agar medium containing glucose or galactose (1 per cent) or sucrose (2 per cent). Beijerinck (1904) saw that *C. variegata* had a great tendency to produce chlorotic cells when cultivated on agar plates that were relatively rich in organic carbon sources such as sugar. When only inorganic carbon sources were available, the cultures remained green when illuminated.

The effect of sugar is dependent on its concentration. Palladin (1918) showed that certain etiolated leaves floated on solutions of low and medium concentrations of sucrose produced chlorophyll when illuminated but failed to do so when the sucrose concentration was 35 per cent. When returned to solutions of 5–10 per cent sucrose concentration, these same leaves greened readily. Trebitz (1905) injected etiolated leaves with sugar solutions of various concentrations and found that with 15 per cent solutions there was no inhibition of greening; with 20 per cent, a visible inhibition occurred; and with 30 per cent (40 per cent for cereals) there was no greening before death. *Chlorella luteo-viridis*, according to Kufferath (1913), remains completely chlorotic in nutrient media containing 7–20 per cent sucrose even though it grows abundantly; with concentrations of 4–6 per cent it produces variegated cultures of yellow and green cells; but with concentrations of 3 per cent or less it forms completely green cultures.

Chodat (1911, p. 515) considers that the chlorosis induced by an excess of assimilable nutrients may arise from a tendency to saprophytism or obligatory parasitism.

Although many substances inhibit chlorophyll formation, the result of their action does not come from a single cause. Some act through hindering chloroplast development, some by action on specific enzymes, and others through "osmotic effects" or inducing "saprophytism"—whatever these last terms mean in this connection.

## 7. CHLOROPHYLL FORMATION IN THE DARK

Up to the present we have been discussing almost entirely chlorophyll formation in the angiosperms, which requires light. In other plant groups, chlorophyll can be formed in the dark. This was discovered by Sachs (1859) when he observed that pine seeds, even though germinated in the dark, produced seedlings with intensely green cotyledons. Later many lower plants were found to produce chlorophyll in the dark. Because of this, Schimper (1885, p. 159) asserted that probably in all the lower forms of plants, up to and including the mosses, the ability to form chlorophyll is independent of light. Bittner (1905) questioned this broad generalization because of the known exceptions; however, she recognized that, in the more highly organized plant forms, the ability to form chlorophyll in the dark is often lost. But, as we will see, this ability is often lost also in the lower forms, such as algae. Although the phylogenetic aspects of this subject present many interesting possibilities for further investigation, they cannot be entered into here. Rather we will discuss some of the physiological aspects that have received attention.

The question whether algae can grow continuously in the dark for long periods of time and produce chlorophyll has been studied by Artari and

by Dangeard. Artari (1902) cultivated *Stichococcus bacillaris* for 4 years, and Dangeard (1921) cultivated *Scenedesmus acutus* for 8 years in the dark without the organisms' losing their green color. During their sojourn in the dark the organisms were frequently transferred to fresh culture media.

Of course, when organisms are grown in the dark, they must be supplied with an organic source of carbon and suitable nitrogenous constituents. Considerable effort has been spent in determining the nutritional requirements for growing algae in the dark and yet maintaining their green color (see Oltmanns, 1923; Kufferath, 1913; Ludwig, 1938). Under favorable nutritional conditions many algae grow and maintain their green color in the dark. There are others, however, e.g., *Chlorella variiegata* (Beijerinck, 1904), *C. luteo-viridis* (Kufferath, 1913), *C. vulgaris* (Finkle *et al.*, 1950), and several species of *Euglena* (Pringsheim, 1948-1949), which readily lose their chlorophyll in the dark. That such organisms lack some specific substance essential for chlorophyll formation which they cannot produce in the absence of light is suggested by the feeding experiments of Pallares *et al.* (1945). These workers discovered that *E. viridis* required the addition of limited quantities of vitamins C and H in addition to the usual organic nutrients in order for them to maintain their green color.

The chlorophylls produced in darkness are the same as those produced in the light. The following organisms have been examined with respect to this property: *Scenedesmus acutus* (Dangeard, 1921), *Chlorella vulgaris* (Radais, 1900; Myers, 1940), *C. pyrenoidosa* (Van Hille, 1938), *Nostoc punctiforme* (Etard and Bouilhac, 1898), and *Protococcus* sp. (Myers, 1940). Myers demonstrated, in particular, the presence of chlorophyll b both by spectrographic and by chromatographic methods. He stated that the ratio of chlorophyll a to chlorophyll b is of the same order of magnitude in cells grown in the light and in the dark.

The physiology of dark-grown pine seedlings is very instructive in regard to chlorophyll formation. The researches of Schmidt and of Bogorad have special significance. Schmidt (1924) demonstrated the close relation between embryo and endosperm in the production of green cotyledons. He separated the white embryos from the endosperms of *Pinus sylvestris* and placed them on moistened filter papers at 27°C in a dark room for 4 days. The embryos grew from 3-mm length to 6 mm but showed no trace of chlorophyll by visual or spectroscopic examination. The embryos when placed in diffuse daylight for 5 days became distinctly green. Embryos of other species, *P. strobus*, *P. pinaster*, *P. jeffreyi*, and *Biota orientalis*, behaved similarly. The embryos grew, so that it was not the lack of vitality which hindered greening.

When the embryos were left in contact with only a small piece of endosperm, they greened. Any part of the embryo left in contact with

the endosperm caused greening of the embryo, but contact with the cotyledons was most effective. The greater the surface of contact, the more intensive the greening. Embryos laid on the endosperm greened more on the side of contact than on the other side; and the greater the length of time the embryo and endosperm were left in contact after germination, the greater was the intensiveness of the greening. Embryos removed from their own endosperms and placed on other endosperms of their own species or a different species still formed chlorophyll in the dark. From these results, Schmidt concluded that a chlorophyll agent ("Chlorophyll-Agens") is transferred from endosperm to embryo which transforms the chlorophyllogen of the embryo to chlorophyll. But attempts to get greening with disintegrated endosperms were fruitless.

Bogorad (1950a) investigated the greening of *P. jeffreyi* seedlings in the dark and found that extirpated embryos from ungerminated seeds did not form chlorophyll in the dark either on synthetic media or on various extracts of endosperms; that illumination of the germinated embryo caused greening; and that, within limits, seedlings synthesized chlorophyll more rapidly and in greater amount, the longer they had been in contact with the megagametophyte after germination. Embryos excised from the endosperm at different stages of greening continued to form chlorophyll. The rate of chlorophyll formation in the germinating embryo after extirpation increased up to the eighth day of contact with the megagametophyte and then decreased. Embryos that have been greened by illumination lose part of their chlorophyll during storage in the dark.

Bogorad's results demonstrated that the amount of chlorophyll formed in darkness by the unextirpated embryo was equal to the amount of chlorophyll formed in the extirpated embryo by illumination (*ibid.*, p. 227). From this quantitative correspondence of the amount of chlorophyll produced, it appears that the chlorophyll precursor is present in the embryo and is converted to chlorophyll either by a substance transferred from the endosperm or by action of light.

In an attempt to determine the nature of the chlorophyll agent, Bogorad followed the transfer of material from the megagametophyte to the sporophyte. He observed the transfer of organic matter, iron, and both inorganic and organic compounds of magnesium, but he could obtain no evidence as to its nature. Although magnesium compounds were transferred, the magnesium in the chlorophyll of the sporophyte never exceeded the magnesium initially present in it. It is unlikely that a holoenzyme is transferred from endosperm to embryo.

The chlorophyll in conifer seedlings grown in the dark has the same absorption bands as that formed in plants in the light (Schmidt, 1924). Smith and Koski (1947-1948) found the ratios of chlorophyll a to chlorophyll b to be 3 to 1 for *Pinus coulteri* and 3.7 to 1 for *P. jeffreyi*. For

the latter, Bogorad reported a ratio of 2.4 to 1. Protochlorophyll has been detected in dark-grown seedlings of *Larix europaea* and *Thuja occidentalis* by Lubimenko (1928) and in *P. coulteri* and *P. jeffreyi* by Smith and Koski (1947-1948). In the two last-named plants the ratio of protochlorophyll to chlorophyll a is about 1 to 50.

Although the cotyledons of conifers are green when grown in the dark, new leaves grown on mature branches and saplings placed in the dark are almost completely devoid of chlorophyll (Boehm, 1859; Frank, 1870, cited by Stahl, 1909; Lubimenko, 1926; Smith and Koski, 1947-1948). Whatever chlorophyll they contain is a mixture of chlorophylls a and b (Smith and Koski, 1947-1948). They contain protochlorophyll (Liro, 1911; Smith and Koski, 1947-1948). These etiolated needles green only very slowly when placed in diffuse daylight at favorable temperatures (Lubimenko, 1926; Smith and Koski, 1947-1948).

Greening in the dark depends on the temperature. Boehm (1863) found that seeds of *P. pinca* held at room temperature long enough to start germination when placed in the dark at 5°-7° Réaumur were completely etiolated. The species differ in their response to temperature: some are completely etiolated, whereas others become slightly green when grown at low temperatures. They green almost universally in the light at these same low temperatures (Boehm, 1865; Burgerstein, 1900). Bogorad (1950a) has shown that the quantity of chlorophyll accumulated in complete seedlings of *P. jeffreyi* in the dark at 34° or 37°C is much less than at 23.5°C.

Schmidt (1924) determined that the red-to-green part of the visible spectrum caused greening in *P. silvestri* embryos much more readily than the green-to-violet portion did.

There are a large number of other plants that green in the dark for which only fragmentary data concerning their physiological behavior are available. For information on these plants, the original articles must be consulted (Bittner, 1905; Liro, 1911; Schimper, 1885; Burgerstein, 1900; Lubimenko, 1926, 1928).

## 8. USE OF CHLOROPHYLL MUTANTS

Frequently, among the higher plants, individuals appear which are abnormal in respect to their chlorophyll: some plants are completely white, some are yellow, and some possess bizarre variegated patterns. These chlorophyll mutants furnish material that can be of service in determining the processes involved in the biosynthesis of chlorophyll and in its accumulation.

Corn, for example, possesses over a hundred specific genetic factors affecting chlorophyll (cf. Emerson *et al.*, 1935; Demerec, 1935). Several of the chlorophyll-deficient mutants of corn have been examined in order

to determine some of the factors that govern their chlorophyll deficiencies. All the mutants examined produce protochlorophyll in the dark, but in various quantities (Fig. 7-20; cf. Table 7-3). All the mutants but one, white seedling-3, produce less protochlorophyll than does the normal sib. A 5-min period of irradiation of the mutant seedlings converts their protochlorophyll to chlorophyll a, and to about the same extent. But a 6-hr period of irradiation affects the chlorophyll content of the mutants differently: it increases the chlorophyll content of the normal seedling

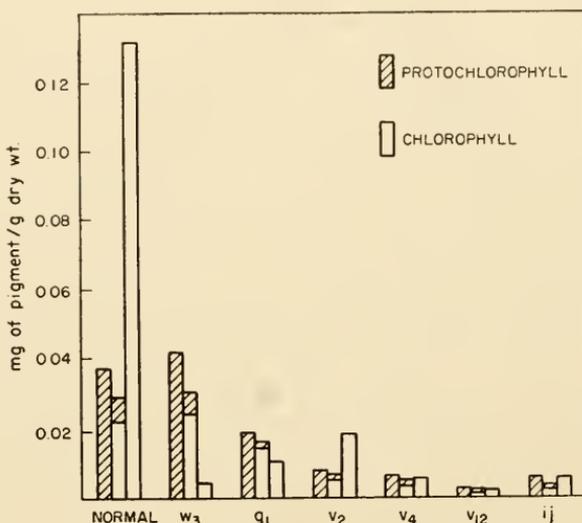


Fig. 7-20. The transformation of protochlorophyll to chlorophyll a and the accumulation or destruction of chlorophyll in various mutant strains of corn. The left-hand rectangle of each set shows the protochlorophyll content of the dark-grown seedlings; the center rectangle shows the protochlorophyll (hatched) and the chlorophyll a (unhatched) content after a 5-min illumination; the right-hand rectangle gives the chlorophyll a content after a 6-hr illumination. (Koski, 1949.)

about sixfold and of the mutants  $v_2$ ,  $v_4$ ,  $v_{12}$ , and  $ij$  to lesser degrees, but it decreases the chlorophyll content of  $g_1$  slightly and of  $w_3$  almost to the vanishing point.

Thus examination of chlorophyll-deficient mutants has demonstrated that chlorophyll deficiency is effected in two ways: by insufficient production of chlorophyll precursor, protochlorophyll; and by heightened chlorophyll destruction.

A study of the chlorophyll mutants of barley by Highkin (1950) has shown that a mutant (chlorina stock No. 2) can exist which contains no chlorophyll b. This mutant grew to maturity and produced seed, but it was not so vigorous as its normal sib. Eyster (1924) reported that he had obtained chlorophyll mutants of corn which contained either only chlorophyll a or chlorophyll b. Mutants of neither type matured.

These observations suggest that the formations of chlorophylls a and b are independent processes. Schwartz (1949) has found that corn mutants vary with respect to the rate at which chlorophyll b appears subsequent to the appearance of chlorophyll a. In one luteus mutant "chlorophyll a preceded the synthesis of chlorophyll b by 4 days when grown at 85°F. . . . At 62°F the retardation extended over a period of 11-14 days."

Granick (1951) has summarized his effective use of biochemical mutants to signalize the path of biogenesis of chlorophyll. By X irradiation he has obtained several *Chlorella* mutants, each of which, in the dark, accumulates a characteristic pigment closely related chemically to protochlorophyll. In the order of their closeness to protochlorophyll, these pigments are magnesium vinylpheoporphyrin-a<sub>5</sub> (protochlorophyll minus phytol) (Granick, 1950), magnesium protoporphyrin (Granick, 1948b), and protoporphyrin-9 (Granick, 1948a).

These mutants vary in their response to irradiation. The mutant colonies containing the magnesium protoporphyrin "developed on the inorganic salts-glucose agar medium had a dull yellow color which turned orange-brown in 4 to 7 days when grown either in the light or dark at room temperature" (*ibid.*). As for the mutant cells that contain magnesium vinylpheoporphyrin, "When grown in the dark on a solid medium of agar, glucose, and inorganic salts, . . . they form deep yellow colonies tinged faintly greenish. . . . When grown in the light, the cells become deep green. . . . In the light these mutants behave like wild type *Chlorella*, growing in the absence of glucose, i.e., photosynthesizing. . . . In contrast to the wild type, this yellow *Chlorella* mutant resembles the higher plants in requiring light for the production of chlorophyll" (Granick, 1950).

## 9. MECHANISM OF CHLOROPHYLL BIOSYNTHESIS

The various schemes proposed for the biosynthesis of chlorophyll have been summarized by Rothemund (1935) and by Aronoff (1950). Many of them are of only historical interest, and no attempt will be made here to discuss them further. Rather, we will try to draw from the facts just presented as full and as up-to-date a picture of this process as is possible.

It is clear that protochlorophyll is the immediate precursor of chlorophyll a: its transformation to chlorophyll a is very rapid and almost complete; its conversion is a molecule-for-molecule reaction; and the action spectrum for its transmutation to chlorophyll a closely resembles its absorption spectrum in organic solvents, provided allowance is made for its absorption in the holochromatic state.

The chemistry of protochlorophyll and chlorophyll, as far as is known, indicates that the reaction is a hydrogenation of protochlorophyll—effected by light in the higher plants. From what source the hydrogen

is derived is not known, but it is unlikely that it comes directly from water, since no oxygen is evolved in the transformation (Smith, 1950-1951).

In the angiosperms the transformation of protochlorophyll is controlled by photochemical action. This is demonstrated by the low temperature coefficient for the photochemical transformation, by the proportionality of the transformation to the light intensity, and by the nature of the action spectrum.

Whether the photochemical process is also enzymatically controlled is not known. The photochemical transformation does not take place in organic solvent extracts or protochlorophyll, in etiolated leaves killed by hot-water immersion, or in a purée of fresh etiolated leaves (Scharfnagel, 1931; Noack, 1934). It is reported to take place, however, in frozen etiolated leaves and in dried leaves (Liro, 1908)—about which there is some doubt (Eyster, 1928; Scharfnagel, 1931)—and in etiolated leaves treated with cyanide. These observations are ambiguous concerning the direct involvement of enzymes in the photochemical reaction, but they suggest that the photochemical reaction can occur as long as the holochrome is intact.

The demonstration that under favorable temperature conditions leaves repeatedly form protochlorophyll and transform it to chlorophyll when placed alternately in the dark and in the light brings convincing evidence that protochlorophyll is the precursor of chlorophyll in the over-all process of greening. This effect substantiates the assumption that the greening process is the result of the continuous thermochemical formation of protochlorophyll and its photochemical transformation to chlorophyll.

The thermochemical reaction is undoubtedly controlled by enzymic processes, because the over-all greening is hindered by unfavorable temperatures both low and high and by the action of enzyme poisons.

In the greening of higher plants both chlorophylls a and b accumulate. It has been proposed that chlorophyll b is derived from chlorophyll a (Rudolph, 1934). Since chlorophyll b appears subsequently to chlorophyll a in the course of chlorophyll accumulation, this assumption is plausible. However, once chlorophyll b is formed, the two chlorophylls accumulate in direct proportion to each other, and the ratio of the rates of formation is little affected by temperature or by intermittency of illumination (Fig. 7-18). If chlorophyll b were formed from chlorophyll a by thermochemical action, its proportion should be greatly increased by the use of intermittent illumination; if it were formed from chlorophyll a by photochemical action, it should increase at a rate proportional to the concentration of chlorophyll a; and if it were in rapid, mobile equilibrium with chlorophyll a, it should be formed at an earlier stage during intermittent illumination than during continuous illumination. None of these propositions is true. Therefore it is more reasonable to assume that chlorophylls a and b are formed concurrently from some common pre-

cursor than to assume that chlorophyll b is formed from chlorophyll a [Smith (1949b); cf. Seybold (1942); for criticism of this hypothesis see Aronoff (1950)].

To account for the formation of the two chlorophylls, Seybold (1948-1949) has suggested the possible participation of two protochlorophylls, protochlorophylls a and b. It is entirely possible that protochlorophyll b is formed prior to chlorophyll b, but so far no evidence for the existence of the b component in dark-grown unilluminated seedlings of the angiosperms has been presented, and its absence is attested by the failure of chlorophyll b to be formed in the initial stages of illumination of etiolated leaves.

The greening of conifer seedlings in the dark has been ascribed to the action of a "chlorophyll agent." Although it is reasonable to hypothesize that this substance is formed in the endosperms of germinating seedlings and diffuses into the embryos, thereby causing them to green, it is not excluded that, at the surface of contact between the two organs, a substance is formed which diffuses into the embryo and causes it to green.

But illumination of the extirpated embryo also brings about greening. The question then arises whether illumination produces a "chlorophyll agent" and thereby indirectly causes greening or whether light acts directly on the chlorophyll precursor to cause greening. Since the conifer seedlings do not continue to green when returned to darkness and since the effect of light is distinctly localized in the angiosperms, it seems very doubtful that light acts indirectly in greening by producing a "chlorophyll agent." It is much more likely that light acts directly on the chlorophyll precursor.

There seems to be no essential difference in the chlorophylls formed or in the nature of the transformations accomplished in the dark and in the light, because in the conifer seedlings protochlorophyll, chlorophyll a, and chlorophyll b have all been identified.

Now we come to the precursors of protochlorophyll. Little is actually known of these in the direct series of reactions leading to protochlorophyll.

Only a limited quantity of protochlorophyll is accumulated in dark-grown leaves before they are illuminated and in leaves that are returned to the dark after they have been illuminated for a brief period. Noack (1934) has suggested that this limit results from an equilibrium or steady-state condition between protochlorophyll and its precursors; that by a short irradiation of the leaves protochlorophyll is removed to form chlorophyll; and that, when the leaves are returned to the dark, the equilibrium condition is restored by the formation of more protochlorophyll from its precursors.

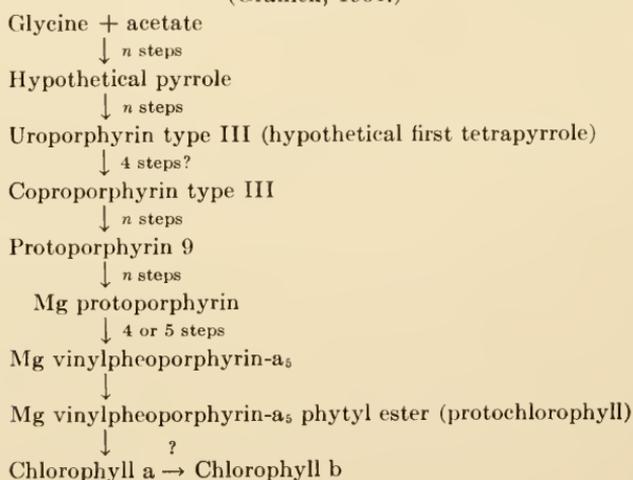
An alternative possibility also exists: that illumination of the dark-grown leaves, besides converting protochlorophyll to chlorophyll, simultaneously produces a precursor of protochlorophyll which is transformed

to protochlorophyll in the dark by thermochemical reactions. This mechanism is suggested by the formation of magnesium-containing compounds through irradiation of etiolated leaves at low temperature (cf. Fig. 7-8).

We are indebted to Granick for much of whatever detailed knowledge we have of the precursors of protochlorophyll. His isolation of protoporphyrin-9 from X-ray-induced mutants of *Chlorella* has given evidence for the similarity of the biosynthesis of porphyrins in animals and plants (Granick, 1951). Since the biosynthesis of protoporphyrin-9 in animals has been shown to come entirely from glycine and acetate molecules "through a compound arising from the tricarboxylic acid cycle" (Shemin and Wittenberg, 1951), it may be presumed that the porphyrins in plants are derived by the same metabolic path. This hypothesis has been made more reasonable by the observation of Salomon *et al.* (1950), who showed by the use of tracers that *C. vulgaris* "is able to utilize directly the alpha carbon atoms of glycine and acetate for the biosynthesis of chlorophyll."

On the basis of the tracer studies and the compounds isolated from *Chlorella* mutants—protoporphyrin-9, magnesium protoporphyrin, and magnesium vinylpheoporphyrin- $a_5$ —Granick (1948-1949, 1951) has proposed a rational sequence for the biosynthesis of protochlorophyll and chlorophyll a (Table 7-7).

TABLE 7-7. A SCHEME FOR THE BIOSYNTHESIS OF CHLOROPHYLL  
(Granick, 1951.)



From the authors' own experience it is proposed that, in those plants which form chlorophyll only in the light, some of the intermediate reactions prior to protochlorophyll formation are light-induced, particularly those associated with the incorporation of magnesium into organic combination; also that chlorophyll b does not arise from chlorophyll a but comes from a precursor, probably preceding protochlorophyll, which is

common to both chlorophylls. A specific proposal for the inclusion of these concepts would be premature at the present time.

But in addition to and beyond the biosynthetic steps in the formation of chlorophyll, there are other factors that must be considered relative to chlorophyll accumulation. The work on the genetic mutants of corn has demonstrated that not only must sufficient precursor be formed to give normal greening but that conditions must be favorable for the protection, and consequent accumulation, of chlorophyll once it has been formed. Albinism probably is not so much the result of a "genetic block" in the chain of biosynthetic reactions leading to the formation of chlorophyll as it is a disturbance of factors that protect the chlorophyll already synthesized.

There is a close connection between the biosynthesis of chlorophyll and the protoplasm in which it arises. Boehm clearly recognized this and articulated it in his name for chlorophyll precursor—"chlorophor." The interconnection between the biological system and the biosynthesis of chlorophyll has been demonstrated by the action of inhibitors on chlorophyll formation, particularly by the use of streptomycin. This inhibitor acts through the hindering of chloroplast formation. When the chloroplasts cannot become organized, chlorophyll formation fails.

If this review has any virtue, perhaps it resides in highlighting the many unanswered questions remaining in fields that have already been explored and in pointing out whole areas that remain unexplored. The organic and physical chemistry of the extracted chlorophylls has advanced far beyond the biochemistry and physiology of the holochromatic pigments. The investigation of the vital reactions, not merely of the two best-known chlorophylls but of all of them, will bring rich rewards in the foreseeable future. •

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For the chemistry of chlorophyll and for the relation of chlorophyll to photosynthesis, the reader is referred especially to Willstätter and Stoll (1913, 1918), Stoll and Wiedemann (1938), Fischer and Stern (1943), and Rabinowitch (1945, 1951).

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## Nitrate Reduction

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*General. Indirect actions of light on the nitrate reduction. Indications of a direct light action. Theories. Addendum. References.*

### 1. GENERAL

A reduction of nitrate to nitrogen at lower levels of oxidation occurs in most plants and is probably lacking only in heterotrophically specialized bacteria and fungi. The process is, nevertheless, imperfectly known biochemically. There are, apparently, at least two different ways in which nitrate reduction occurs: One is the usually anaerobic reduction of nitrate to nitrite in the bacterial denitrification, in which the nitrate serves as an oxygen donor for the respiration; another is the reduction that forms an integral part of the assimilation of nitrate in all higher plants and many bacteria and fungi. The bacterial denitrification is undoubtedly a process that takes place in darkness independently of light, whereas the assimilation of nitrate in many instances has been shown to be intimately dependent upon the light climate of the plant.

The bulk end product of the utilization of nitrate is amino nitrogen, and the process may be simply expressed as  $\text{HNO}_3 \rightarrow \text{RCH}_2\text{NH}_2$ . Thus it involves the reduction of nitrogen from the  $\text{N}_2\text{O}_5$  to the  $\text{NH}_3$  level and its binding in an organic linkage. The reduction must, of course, take place in several steps, but it is not definitely known at which level of oxidation the nitrogen is assimilated (Burström, 1945). It has been shown with fungi (Kostytschew and Tswetkova, 1920) and bacteria (Burris and Wilson, 1946) that ammonia appears as a direct product of reduction; nevertheless this does not exclude the possibility that the nitrogen is fixed in an organic linkage at a higher level of oxidation. As to higher plants, it is less probable that nitrate is reduced down to the ammonia level before it is assimilated (Burström, 1945). This is especially true of chlorophyllous parts of plants (cf. Lemoigne *et al.*, 1936, 1937, 1938), in which the utilization of nitrate takes place preferably in the light.

This uncertainty concerning the connection between reduction and

assimilation renders a chemical formulation of the nitrate reduction impossible. Thus it is necessary for the following discussion to exclude the assimilation in the restricted sense and to regard the nitrate reduction preliminarily as an entirely inorganic reaction leading to ammonia,



with the reservation that  $\text{NH}_3$  actually stands for nitrogen at this level of oxidation in the organic or inorganic form.

No appreciable amounts of inorganic or organic intermediary products of the nitrate reduction appear normally or have been shown to appear under ordinary conditions in higher plants, but the reduction schematically expressed by Eq. (8-1) practically equals the consumption of nitrate. Most plants, however, are able to accumulate nitrate in the unreduced form in larger or smaller amounts, and therefore the consumption cannot simply be considered equal to the absorption of nitrate from the external nutrient medium, a circumstance that must not be overlooked.

It has long been known that the consumption of nitrate in the green parts of plants is considerably accelerated by light. Ever since Schimper's work (1888), this fact has given rise to discussions of a possible direct participation of light in the reduction of nitrate, perhaps analogous to the function of light in the photosynthetic assimilation of carbon dioxide. This possibility has been denied as often as it has been confirmed, or perhaps more often. There are, however, surprisingly few reliable quantitative data in the literature directly illustrating the consumption of nitrate in relation to the light conditions which can be brought forward as conclusive arguments in this discussion. They will be dealt with in detail in Sect. 3.

Diurnal fluctuations of the nitrate content of green leaves have been observed by Chibnall (1922); bean leaves contained 0.019 per cent nitrate in the evening, and in the night the figure rose to 0.025 per cent. However, such figures can by no means be interpreted simply as an increase in the nitrate content in the dark and a consumption of nitrate in the daytime. A more complete picture has been obtained with leaves of *Helianthus annuus* in experiments (unpublished) carried out at the author's institute by H. Rufelt (Fig. 8-1). These observations were made under ecological conditions, i.e., with a normal periodicity of the meteorological factors. This causes, of course, some irregularity in the diurnal variation of the nitrate content. The example recorded in the figure shows a distinct minimum of the nitrate content after noon, which seems to recur regularly. Another was observed 12 hr later, before dawn, so that the nitrate content showed one maximum after sunset and another in the morning. Thus the periodicity does not follow to any appreciable extent the change between light and darkness.

The amount of nitrate accumulated at any moment is only the net

result of supply and consumption, depending upon such factors as the rate of photosynthesis and carbohydrate formation and the rate of migration of nutrients to the leaves, both partly regulated by the stomatal movements, the temperature, etc., apart from the hypothetical direct action of light on the nitrate reduction. Thus there are several ways in which light regulates the nitrate consumption indirectly, and three of them deserve special attention.

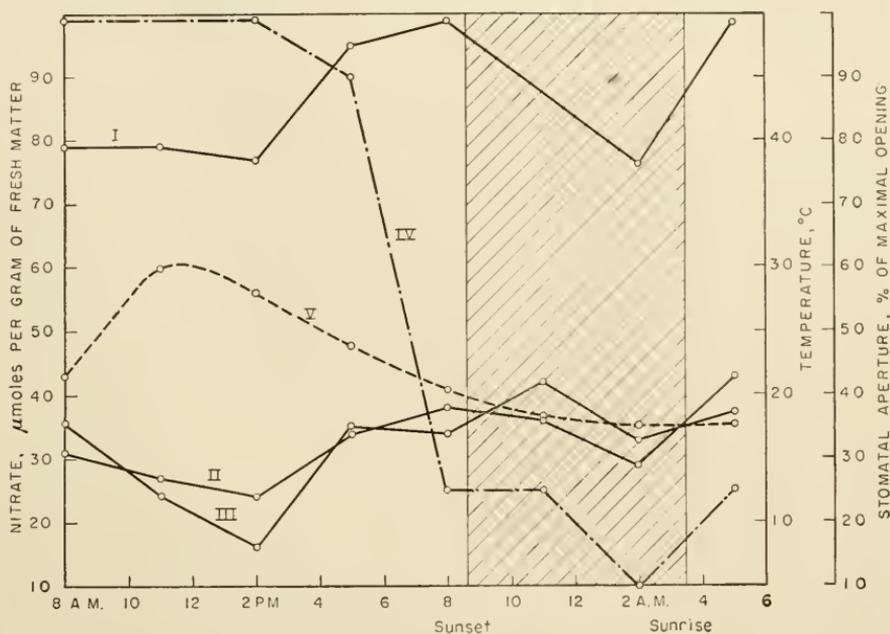


FIG. 8-1. The diurnal variation in nitrate content of *Helianthus annuus* (July 10 and 11, 1945). I, nitrate content in stems; II, nitrate content in old leaves; III, nitrate content in young leaves; IV, stomatal aperture, percentage of the maximal opening; V, air temperature. Nitrate-content curves read on left ordinate; stomata-aperture and air-temperature curves, on their respective ordinates at left. (Experiments by H. Rufelt, unpublished.)

## 2. INDIRECT ACTIONS OF LIGHT ON THE NITRATE REDUCTION

2-1. *The Stomatal Factor.* An important action of light is indicated in Fig. 8-1 as the stomatal aperture, which shows a common kind of diurnal periodicity with closure in the afternoon and opening after sunrise. If the less important variations of temperature and humidity are neglected, the transpiration should roughly follow the course of the stomatal movements. It has further been shown that, with all other conditions constant and the plants adapted to the particular external nutrient medium, the absorption of ions and their transport to the leaves closely follow the rate of transpiration (Hylmö, 1953). The supply of nitrate to the leaves

in the morning until 2 P.M. (Fig. 8-1) is consequently high, and the comparatively slight reduction of the nitrate content at this time of the day must correspond to a considerably higher rate of nitrate consumption than in the evening with closed stomata and, nevertheless, increasing content of nitrate. The importance of the stomatal movements for the nitrate consumption is obvious. The cause of the second minimum of the nitrate content around 2 A.M. has not been disclosed. It coincides with the minimum of stomatal aperture and may illustrate some other factor that ought to be considered for a full interpretation of the diurnal rhythm.

The effect of the stomatal factor on the transpiration was eliminated by Dittrich (1930) in experiments with wheat leaves. In leaves kept in ordinary dry air, the nitrate content increased between 4 and 11 A.M. from 1.86 to 2.16 mg per gram of fresh matter; if, however, the transpiration under the same conditions was kept down by spraying the plants with water, the content of nitrate decreased from 1.52 to 1.29 mg/g. The increase in the nitrate content observed in this instance in the morning can accordingly be ascribed to the transpiration. From 11 A.M. to 7 P.M., on the contrary, the nitrate content in ordinarily treated leaves decreased from 2.16 to 1.30 mg/g, and in water-sprayed leaves, from 1.29 to 0.88 mg/g. This decrease obviously cannot depend upon a change in transpiration and the ensuing nitrate supply but must involve a consumption of nitrate. It may, as Dittrich has suggested, indicate a direct light action on the nitrate reduction; there is, however, still to be considered the importance of the carbohydrate metabolism for the nitrate consumption, and even here the stomatal movement may be the decisive factor.

A more reliable method of eliminating the transpiration and the absorption of nitrate is to study the reduction of nitrate accumulated in leaves in its relation to light, provided that a migration of nitrate within the leaf does not limit the reduction. Such experiments have likewise been carried out with wheat leaves (Burström, 1937, 1943a,b), and they have shown the reduction to be much more intimately connected with the illumination than would be expected from the rather slight diurnal variations of the nitrate content under ecological conditions. The reason, as has been exemplified in the foregoing, is that an increased absorption in the light seemingly counteracts the increased consumption. In one pair of experiments (Burström, 1937), for instance, the assimilation in the light amounted in 48 hr to 0.084 mmole of an available 0.240 mmole, or 35 per cent, whereas in darkness it did not exceed 7 per cent.

Repeated experiments of this kind (Burström, 1943a,b) have failed to disclose any significant reduction of nitrate in wheat leaves in darkness, but they show a measurable consumption even at fairly low light intensities, as will be discussed fully in the next section. Even with this

method, careful attention must be paid to the carbohydrate factor before any conclusions can be drawn as to a direct light effect on the nitrate consumption.

It is also premature to generalize from this result, because other plants may behave differently. There is, as a matter of fact, only one other green plant extensively studied in this respect, the alga *Chlorella*, and it does not behave at all in this manner. *Chlorella* can undoubtedly assimilate nitrate in darkness (Warburg and Negelein, 1920; Myers, 1949), but the rate of assimilation appears to be considerably increased in the light.

The light effect in *Chlorella* has been ascribed to either one of two indirect actions: the permeability or, as usual, the carbohydrate metabolism, besides a direct action on the nitrate reduction.

2-2. *The Permeability Factor.* The influence of light on the permeability in connection with general structural features of the cytoplasm is dealt with by Stålfelt in Chap. 12, and reference is made to this treatise for the general background of the problem. If there is an increase in the permeability of the cytoplasm in the light, this may influence the migration of compounds into or between cells and affect the rate of metabolism disguised as a light effect on the reactions themselves. This circumstance must always be considered in interpreting light effects on intact cells. Warburg and Negelein (1920), the first to investigate the nitrate reduction in *Chlorella*, computed from the gas exchange an at least threefold increase in the nitrate consumption in the light. They assumed, apparently for lack of other possibilities, that this depended upon an increased uptake of nitrate, referring to the light-permeability relation. With regard to the later development of the conception of the active absorption of ions, this explanation is hardly convincing without more positive evidence. It is pertinent to mention in this connection that Pearsall and Billimoria (1939), with excised pieces of *Narcissus* leaves, found an increased absorption of nitrate in the light which they attributed to a rise in the temperature. This was not much more than a supposition. However, Pearsall and Billimoria were able to eliminate the carbohydrate factor in the nitrate assimilation by amply supplying the leaves with sugar, but, in spite of this, synthesis of protein took place only in the light. An effect of light on the intake of nitrate by cells may contribute to the light induction of the reduction, but it can scarcely explain the entire phenomenon.

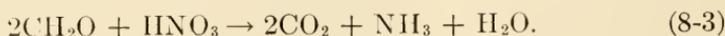
2-3. *The Carbohydrate Factor.* Since the final products of the assimilation of nitrate are amino compounds, a supply of nitrogen-free organic matter is necessary for the process. In the dark this means that the assimilation takes place at the expense of stored carbohydrates.

When the reduction is expressed as in Eq. (8-1), it implies the presence of a hydrogen donor. If we still consider only the conditions in the dark, the only source of hydrogen is probably the glycolytic breakdown of

sugar. This means that carbohydrates are necessary for the reduction, apart from their importance as sources of carbon for the ensuing or simultaneous synthesis of organic nitrogen compounds. If the breakdown of sugar is written



Eqs. (8-1) and (8-2) can be summed up to



The presence of carbohydrates and the respiratory breakdown of sugars will under all circumstances in darkness become prerequisites for the reduction of nitrate; hence the supply of carbohydrates must always be considered an important factor regulating the rate of the process. Irrespective of the details of the connection between nitrate reduction and respiration, the result according to Eq. (8-3) implies a production of carbon dioxide without a corresponding uptake of oxygen, or a formation of "extra carbon dioxide," according to Warburg and Negelein (1920). Their discovery of an extra carbon dioxide formation in *Chlorella* in the presence of nitrate showed that the reduction takes place according to the general principles expressed in formula (8-3), and this has been corroborated in later investigations (Ruhland and Ullrich, 1929; Tamiya, 1932; Yamagata, 1934; Myers and Cramer, 1948).

Other results have been obtained in the light, but their interpretation requires a consideration of the significance of the dark reaction, Eq. (8-3). The first question is whether this formula actually holds true.

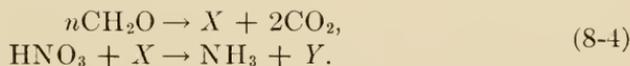
The weakest point in this discussion is that in no instance have both the consumption of nitrate and the production of extra carbon dioxide been determined in the same short-time experiment. Warburg and Negelein determined the production of ammonia and put it in relation to the production of extra carbon dioxide. Theoretically the ammonia/carbon dioxide ratio should be 1/2, but this value was observed only occasionally. The carbon dioxide evolution was initially much higher than was expected; this was reasonably explained by assuming an assimilation of part of the ammonia formed in the reduction. In the course of time the formation of extra carbon dioxide decreased and that of ammonia increased, but every series of experiments was cut off at the point where a carbon dioxide/ammonia ratio of 2/1 was attained. Yamagata and Tamiya computed in experiments of long duration the extra carbon dioxide production in relation to consumed nitrate and found agreement with the theory. This is inevitable, however, according to Yamagata's deductions, if the average elementary composition of the cell matter does not change through the assimilation of nitrate, so that the consistency does not prove the strict validity of Eq. (8-3). The occurrence of an extra carbon dioxide formation is established beyond doubt, but it is an

open question whether it can be quantitatively expressed by the proposed equation, Eq. (8-3). The unsettled problem is how nitrate is linked with the breakdown of sugar and how far it is reduced before it is assimilated.

It ought to be mentioned in this connection that several authors discuss an extra gas formation in darkness or in light from inadequate observations, in so far as they lack determinations of either oxygen or carbon dioxide, but present figures that do not necessarily denote more than an increase in the respiratory intensity (Wehner, 1928; Hamner, 1936; Lovell, 1938; Gilbert and Shive, 1945). Such an increase can, of course, be caused merely by the presence of nitrate or a higher nitrogen content, apart from an extra gas production.

It is, furthermore, of importance to observe that nitrate can hardly function simply as a hydrogen acceptor in the terminal oxidation instead of oxygen, which could be inferred from Eq. (8-1). Thermodynamic objections may be raised against such a simplification of the problem, and there are two physiological reasons as well: First, the addition of nitrate seems to cause a production of extra carbon dioxide mainly by increasing the liberation of carbon dioxide, whereas the consumption of oxygen may remain unchanged or decrease slightly (Ruhland and Ullrich, 1929; Cramer and Myers, 1948). This means that nitrate must increase the glycolytic breakdown of sugar and not simply replace oxygen. It is known that oxygen tensions corresponding to the normal content of the air do not limit the respiration to any great extent. Thus it seems unlikely that an increased supply of hydrogen acceptors can augment the consumption of sugar. Second, the normal assimilation of nitrate, in contrast to the bacterial denitrification, seems to be an aerobic process (Warburg and Negelein, 1920; Yamagata, 1934; Burström, 1939); this does not quite agree with the idea that nitrate can replace oxygen as an oxidizer. Nance (1948, 1950) assumes, on the other hand, that the step nitrate to nitrite is really an anaerobic reaction and that only the subsequent steps are aerobic.

These reflections lead to the conclusion that nitrate can hardly act strictly as assumed in Eqs. (8-1) to (8-3); it seems even more probable, on the contrary, that nitrate reacts with some intermediary product of the glycolysis, presumably at such an early stage of the breakdown of sugar that this is accelerated by nitrate removing the intermediary compound. This may be written



The reduction of nitrate to the ammonia level involves several steps performed with the aid of different hydrogen donors, but only the first one has as yet been successfully studied in green plants. Evans and Nason (1952, 1953) have clearly demonstrated in different plant mate-

rials a reductase that reduces nitrate to nitrite, with hydrogen ions and reduced triphosphopyridine nucleotide (TPNH + H<sup>+</sup>) serving as the hydrogen-donating system [see Eq. (8-9)]. They point out that this explains the connection with the carbohydrate metabolism, which is only partly true. Physiologically it is scarcely possible to separate the initial nitrite formation from the bulk process of reduction and assimilation of nitrate, and it is even likely that nitrite in the free state cannot appear *in vivo* in higher plants as a free intermediate product (Burstrom, 1945), although in some instances it has been detected in minute amounts (e.g., Nance, 1948). Nitrite is not attacked by the reductase system of Evans and Nason. However, carbohydrates or derivatives thereof must be consumed in the whole sequence of reactions leading to amino acids. It is therefore pertinent to present the physiological data that have a bearing on a direct light action on the nitrate reduction and assimilation before returning to the enzyme chemical results, which deal only with the initial reduction to nitrite.

### 3. INDICATIONS OF A DIRECT LIGHT ACTION

There are only two green plants in which the light actions have been studied closely, namely, *Chlorella* and wheat. Since both the materials and the methods have been rather different, it seems appropriate to discuss these two cases separately.

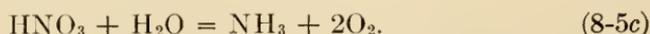
3-1. *Experiments on Chlorella.* The formation of extra carbon dioxide must, of course, cease in the light. Warburg and Negelein (1920) studied the gas exchange of *Chlorella* in light in the presence of nitrate and in the absence of carbon dioxide, finding a respiratory quotient of less than 1. This means that oxygen was produced in excess of the assimilated carbon dioxide. This "extra oxygen" in the light should correspond to the extra carbon dioxide produced in darkness and originate in a normal photochemical assimilation of the extra carbon dioxide. The dark reaction is expressed by Warburg and Negelein by



and the ensuing light reaction by



The net result in light thus becomes



This formation of extra oxygen in the light in *Chlorella* has been confirmed by Myers (1949), Myers and Johnston (1949), Pirson and Wilhelmi (1950), and Davis (1950). The theoretical oxygen/ammonia ratio, according to Eq. (8-5c), should be 2/1. Warburg and Negelein,

however, found values between 2/0.3 and 2/0.5, which means a much lower production of ammonia than was to be expected. This was assumed to depend upon an assimilation of the reduced nitrogen, which thus could not be recovered quantitatively as ammonia. Davis, on the contrary, obtained the stoichiometric ratio 2/1 between extra oxygen and added nitrate, which could verify the net Eq. (8-5c) if it is assumed that nitrate is quantitatively consumed during the time of the experiment. Just as in the dark, this does not prove the validity of the formula so far as a reduction to ammonia is concerned; it only shows that the combination of reduction and assimilation of nitrate in the light leads to formation of cell matter of the same average elementary composition as in the dark. Myers and Johnston are the only investigators who have determined the amount of consumed nitrate. It can be computed from their figures (1949, Table 3) that at low light intensity the ratio between extra oxygen produced and assimilated nitrate amounts to 2.9-3.1, but that at high intensity values of 2.0-2.1 are obtained. These latter are very near the assumed theoretical value, but Myers and Johnston logically explain the difference as a change in the over-all composition of the cellular matter formed.

In other experiments Myers and his collaborators obtained respiratory quotients in light of 0.80 and lower, and Pirson and Wilhelmi obtained around 0.70, so that the production of extra oxygen is established; in these instances neither the consumption of nitrate nor the nature and amount of reduced products formed were determined, so that the nitrate reduction had to be inferred from the shifts in the gas exchange. It is thus apparent that the situation is just the same in regard to the conditions in light as in darkness; there are changes in the gas evolution in the expected direction, but their quantitative relation to the nitrate reduction is open to discussion.

There seems to be one discrepancy in these results with *Chlorella*, however. Warburg and Negelein, using a carbon dioxide-free atmosphere, obtained extra carbon dioxide production in the dark and therefore postulated a correspondingly higher rate of nitrate reduction. Myers states that "in the absence of CO<sub>2</sub>, however, the gas exchange is negligible and there is no evidence of nitrate reduction." Davis has briefly recorded that *Chlorella* in a carbon dioxide-free medium produces extra oxygen and hence assimilates nitrate only in the presence of externally added glucose. The same holds true for the reduction in darkness, according to Myers. The contradictions might be due to different pretreatment and conditions of the plants and could be explained if Warburg's algae were richer in carbohydrates than the others. Such a possibility, however, seems to be inconsistent with the fact that Warburg's algae were enormously overfed on nitrate, having been supplied with 0.1 N nitrate in 0.01 N nitrous acid. This may in itself have caused

the high rate of metabolism in the absence of both carbon dioxide and additional sugars, but it also implies that this represents some slightly abnormal activity of the cells.

It is obvious, for the rest, that reduction of nitrate requires either added sugar or light and, further, that the rate of reduction can be much increased by light. The question is whether this can be explained entirely by an increase in the supply of carbohydrates accelerating the dark reaction, Eq. (8-5a), or whether the light itself takes an active part in the reduction. This may be answered tolerably convincingly in favor of the latter assumption before it is worthwhile to discuss different modes of light actions. Purely theoretical considerations have been made, for example, by Ullrich (1924) and Rabinowitch (1945).

Warburg and Negelein claim to have disproved the hypothesis of a direct action by the observation that phenylurethane, known to inhibit photosynthesis [Eq. (8-5b)], converts the extra oxygen production into an equivalent evolution of extra carbon dioxide. This is certainly an interesting observation, but the artificial experimental conditions mentioned detract from its value. A more hypothetical objection has been made by Rabinowitch (1945) by pointing to the possibility that nitrate reduction might shift from one photosynthetical or photoreductive system in the light to another in the dark when the former is inactivated. A parallel should exist in a shift from photosynthesis to photooxidation (Noack, 1925).

The assumption of at least partly different dark and light systems for the reduction and assimilation of nitrate was put forth by the author (Burstrom, 1945) and had earlier been suggested by Kostytschew (1926). It has received support from Myers's results (1949) with *Chlorella* at low light intensity. Myers pictures two systems, one dark and one light, competing for the nitrate, and the way the reduction takes place should depend upon the carbohydrate content of the cells or, expressed more generally, upon the relation between their carbohydrate content and the illumination. Cells developed at a high light intensity, supposedly rich in carbohydrates, when put in the dark showed a higher rate of respiration and a greater production of extra carbon dioxide than cells grown at a low light intensity (Table 8-1). This is in accordance with the expectations [Eqs. (8-2) and (8-3)] if the content of carbohydrates limits the rate of nitrate assimilation. When transferred to light of low intensities, the two sets of cells behaved differently.

High-carbohydrate cells gave irregular figures for the respiratory quotient down to  $-0.48$ . At the lowest light intensity of 35 ft-c the rare phenomenon of a positive respiratory quotient was encountered, which means that both oxygen and carbon dioxide were given off by the cells. The immediate conclusion drawn by Myers is that, owing to the high content of carbohydrates, the reduction follows the dark pattern. Nitrate is

reduced and assimilated in connection with a respiratory breakdown of carbohydrates, but the light intensity is insufficient for a quantitative reassimilation of the extra carbon dioxide. This is given off, and it exceeds in amount the apparent uptake of carbon dioxide in the photosynthesis; thus both carbon dioxide and oxygen are given off. It seems likely that the gas exchange in the nitrate assimilation and photosynthesis can actually proceed in this manner independently of each other because the cells were supplied with 4 per cent carbon dioxide in the air. This renders an increase in the carbon dioxide assimilation by the

TABLE 8-1. GAS EXCHANGE OF *Chlorella* CELLS RAISED AT LOW AND HIGH LIGHT INTENSITIES  
(Computed from Myers, 1949.)

Light, ft-c	Gas exchange		Extra gas		Real CO <sub>2</sub> assimilation <sup>a</sup>
	O <sub>2</sub>	CO <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	
Low-carbohydrate algae					
0 <sup>b</sup>	-1.42	1.94	....	0.52	0
35	3.43	-2.08	1.35	....	3.50
45	5.35	-3.19	2.16	....	4.61
60	7.14	-4.90	2.24	....	6.32
High-carbohydrate algae					
0 <sup>b</sup>	-2.06	3.28	....	1.22	0
35	1.05	0.69	1.74	....	3.11
45	3.08	-0.97	2.11	....	5.14
60	4.13	-1.98	2.15	....	6.19

<sup>a</sup> For computation see text.

<sup>b</sup> Average values given for light of 0 ft-c intensity.

extra carbon dioxide produced highly improbable near the point of compensation.

In plants low in carbohydrates a fairly constant respiratory quotient of about -0.80 was obtained, indicating, according to Myers, that the reduction of nitrate closely follows the photosynthesis. This interpretation can justify an attempt to compute from Myers's data the assimilation of carbon dioxide separated from the gas exchange caused by the nitrate assimilation and the respiration. The formation of "extra gas," according to Warburg, indicates the intensity of the nitrate assimilation regardless of whether oxygen or carbon dioxide is given off in excess; this also follows from Eqs. (8-5a, b, c). Such figures have been computed in Table 8-1, disregarding the fact that in the absence of nitrate the respiratory quotient does not equal exactly 1, an error that has to be neglected. It is obvious that in both high- and low-carbohydrate plants this value

rises to a nearly constant and, in the two instances, equal figure; whether this depends upon the supply of nitrate cannot be inferred from the data.

The interpretation presupposes that the oxygen values in darkness show the normal respiration, to which the extra carbon dioxide is added. Furthermore in high-carbohydrate plants in the light the extra carbon dioxide that is not assimilated causes a reduction in the apparent uptake of carbon dioxide, but the real assimilation could be computed from the oxygen values (column 6 of Table 8-1). In low-carbohydrate plants, on the contrary, the carbon dioxide values in the light may show the real assimilation, and nitrate causes an additional production of oxygen. The striking result is that the two series of values for the real assimilation of carbon dioxide, computed in different ways for the two sets of plants, tally very well. This was to be expected if the computations are correct, because it is hardly probable, with 4 per cent carbon dioxide in the air, that additional gas exchange could influence the actual rate of carbon dioxide fixation.

It is also obvious that the interpretation of Myers, involving two different mechanisms, alone can explain the surprising fact that both oxygen evolution and carbon dioxide fixation are higher in the low-carbohydrate than in the high-carbohydrate algae—the oxygen values because extra oxygen is produced, and the carbon dioxide values because extra carbon dioxide is produced in the latter algae.

The nature of the photoreaction in the low-light algae is not clear. Myers eliminates the possibility of a photorespiration on the ground that it should not exist in *Chlorella* (cf. Burk *et al.*, 1949). Further experiments of Myers have revealed that nitrogen-deficient cells, which ought to be rich in carbohydrates, have an extra carbon dioxide production in the dark of 1.2 units but, at saturating light intensity, an extra oxygen evolution of 10.3 units. If the nitrate reduction was linked with respiration in this instance, this would imply an eightfold increase in the glycolysis in light, which is incompatible with all experience.

This way of reasoning should, as a matter of fact, exclude every possibility except a connection between nitrate reduction and photolysis if we refrain from assuming an independent photochemical splitting of nitrate. There is, however, no evidence for or against the existence of such a mechanism.

3-2. *Experiments on Wheat.* The investigations by the author (Burstrom, 1943a,b, 1945) have likewise led to the assumption of two paths of nitrate assimilation. In wheat plants they should be locally separated. Assimilation of nitrate takes place in both roots and leaves; in the former it is confined to darkness and is independent of light, and in the leaves it is almost entirely dependent upon light. Only the second process need be considered here.

There is no significant assimilation of accumulated nitrate in wheat

leaves in darkness (Burström, 1943a), but it increases with increased light intensity and attains significant figures already below the point of compensation, when there can hardly be any noticeable improved carbohydrate balance and increase in the respiratory utilization of sugar. The maximal rate of nitrate reduction at saturating light intensity and a normal content of carbon dioxide of the air should, furthermore, correspond to a fivefold increase in glycolysis if the reduction were linked with the dark metabolism of carbohydrates. Just as with *Chlorella*, this seems in itself to exclude the possibility that the rate of the dark assimilation of

TABLE 8-2. THE ASSIMILATION OF CARBON DIOXIDE AND ACCUMULATED NITRATE IN LEAVES AT 3250 FT-C

Nitrate, mmoles <sup>a</sup>		CO <sub>2</sub> assimilated, <sup>b</sup> mmoles	Sugars formed, <sup>b</sup> mmoles	Other assimilates	
Initial	Assimilated			mmoles <sup>b</sup>	C/N ratio
0	0	0.244	0.231	0.013	—
0.056	0.032	0.258	0.224	0.034	6.4
0.105	0.062	0.228	0.153	0.075	7.5
0.158	0.103	0.254	0.142	0.112	6.5

<sup>a</sup> Varying initial content of nitrate.

<sup>b</sup> Computed as the equivalent amount of hexose in millimoles.

nitrate is increased in the light through a more favorable supply of carbohydrates. In the case of wheat, however, there are also more positive evidences of a connection between the nitrate assimilation and the photosynthetic mechanism.

The products of the simultaneous assimilation of carbon dioxide and nitrate are of special interest. In the absence of nitrate (Table 8-2), soluble sugars are formed quantitatively from the assimilated carbon dioxide, but with nitrate present the formation of sugar decreases. Other assimilates must be produced, and this is caused by the simultaneous assimilation of nitrate. The amount of carbon disposed of in this way stands in a fairly fixed ratio to the amount of assimilated nitrate, which must be assumed to be converted into some organic compounds. The ratio between carbon and nitrogen deposited in such non-sugar assimilates depends upon the specific properties of the leaf material in question but is independent of the light intensity. In Table 8-2 the carbon/nitrogen ratio amounts to 6-7, but both higher and lower figures are obtained if leaves of different age are separated (Table 8-3). In mature, nongrowing leaves the carbon/nitrogen ratio of these assimilates is approximately 4, or very near that of amino acids, but in rapidly growing leaves it attains values as high as 15-18. This corresponds, as a matter of fact, to the average composition of the cell matter and apparently means that the simultaneous assimilation of nitrate and carbon

dioxide causes growth by formation of new cell matter. The conditions are very similar to those found by Myers with *Chlorella* and permit the conclusion that in nongrowing leaves nitrogen-containing compounds having the average composition of amino acids appear which ought to be regarded as the first identifiable assimilatory products together with sugars. These nitrogenous compounds form if nitrate is present, and the excess carbon dioxide assimilated is deposited in sugars.

TABLE 8-3. THE ASSIMILATION OF CARBON DIOXIDE AND ACCUMULATED NITRATE IN WHEAT LEAVES OF DIFFERENT AGES

Light, ft-c	Age of leaves	Nitrate assimilated, mmoles	CO <sub>2</sub> assimilated, <sup>a</sup> mmoles	Sugars formed, <sup>a</sup> mmoles	Other assimilates	
					mmoles <sup>a</sup>	C/N ratio
840	Full grown	0.049	0.097	0.053	0.044	5.4
3250	Full grown	0.055	0.266	0.231	0.035	3.8
840	Growing	0.024	0.071	0.003	0.068	17.0
3250	Growing	0.045	0.221	0.084	0.137	18.3

<sup>a</sup> Computed as the equivalent amount of hexose in millimoles.

TABLE 8-4. THE RESPIRATORY BREAKDOWN OF SUGAR AND ASSIMILATION OF ACCUMULATED NITRATE IN WHEAT LEAVES AT LOW CARBON DIOXIDE CONTENT OF THE AIR (0.07 MG/LITER)

Light, ft-c	Sugar consumed, <sup>a</sup> mmoles	CO <sub>2</sub> given off, <sup>b</sup> mmoles	CO <sub>2</sub> assimilated, <sup>c</sup> mmoles	Nitrate assimilated, mmoles	C/N ratio, assimilated
0	0.056	0.056	0	0	
270	0.054	0.038	0.016	0.006	16
820	0.051	0.034	0.017	0.007	15
1670	0.051	0.019	0.032	0.010	19
2800	0.055	0.018	0.037	0.012	18

<sup>a</sup> Initial amount about 0.150 mmole, computed as hexose.

<sup>b</sup> As the equivalent amount of hexose in millimoles.

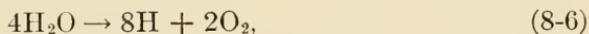
<sup>c</sup> Reassimilated or in other ways converted in non-sugar compounds.

The fact that the respiration is independent of light is easily shown by experiments with a reduced carbon dioxide content of the air [Table 8-4, with 0.07 mg of carbon dioxide per liter; this is close to the apparent threshold value of Gabrielsen (1948), at which there is no net exchange of carbon dioxide with the air]. There is a constant consumption of sugar irrespective of the intensity of the illumination, whereas the assimilation of nitrate steadily increases with the light intensity. In the dark the consumed sugar is quantitatively given off as respiratory carbon dioxide, but in the light it is partly withheld in the leaves as non-sugar compounds. This may be due either to a true reassimilation of respira-

tory carbon dioxide or to a utilization of intermediate products of the respiratory breakdown of sugar. In any case this resynthesis of carbon compounds parallels fairly well the assimilation of nitrate, resulting in a synthesis of compounds containing carbon and nitrogen in a ratio of between 15/1 and 19/1. It seems to have been possible in this instance to separate respiration from photosynthesis and show that the assimilation of nitrate follows the latter. This is rather good evidence of a light-induced reduction and assimilation of nitrate, and, although not conclusive, the results point to a connection between the assimilation of nitrate and carbon dioxide.

#### 4. THEORIES

If we start from the reduction of nitrate expressed as in Eq. (8-1),  $\text{HNO}_3 + 8\text{H} \rightarrow \text{NH}_3 + 3\text{H}_2\text{O}$ , and try to connect it with the photosynthetic mechanism, this is easily performed theoretically by adding the equation of the photolysis,



to the sum



Photolytically formed hydrogen should reduce nitrate to a lower level of oxidation, presumably to ammonia. The arguments in favor of some connection with the photosynthesis have been presented in the foregoing and are rather strong, but there are no arguments in favor of some simple mechanism as expressed in Eq. (8-7).

First, it must be emphasized that we do not know of any photochemical mechanism, other than that of chlorophyll, which can be made responsible for the nitrate reduction. It is true that Tottingham *et al.* (1934) found a special action of visible light of short wave lengths on the utilization of nitrate, but they measured only the nitrate absorption, not the consumption. It is possible that migration effects may have been involved by means of the stomatal mechanism (cf. Sierp, 1933) or in other ways. In any case, the results of Tottingham *et al.*, although interesting and suggestive, form a weak basis for speculations about special photochemical systems for the nitrate utilization.

Second, there are several possibilities even if we assume a connection between nitrate reduction and the general photosynthetic mechanism. Equation (8-7) implies a formation of extra oxygen independently of the transformation of carbon dioxide. The extra oxygen should be added to the one normally produced, and the reduction of carbon dioxide should proceed undisturbedly. This means that the utilization of light energy, as shown by the evolution of oxygen, is increased by a reduction of nitrate together with that of carbon dioxide. Another possibility is that, if the

supply of light energy is limited, nitrate and carbon dioxide compete for the available photolytically formed hydrogen. In the former case the result is an increased production of oxygen, and in the latter, a reduced carbon dioxide assimilation in the presence of nitrate. It should be possible to decide this question experimentally.

Under ordinary conditions, even at a moderate light intensity, the assimilation of carbon dioxide can be increased considerably by increasing the supply of this gas. This means that light under such circumstances does not limit the reductions, and hydrogen ought to be formed photolytically in excess of that which is used for photosynthesis at the normally low content of carbon dioxide in the air. This excess could, of course, be utilized for the reduction of nitrate; ordinarily it must be reoxidized by oxygen to water. If this is the case, the extra oxygen production must, as emphasized by Pirson and Wilhelmi (1950), consist of an increase in the oxygen evolution. They claim that their own experiments have shown a deficit in the consumption of carbon dioxide but admit that "die O<sub>2</sub>-Entwicklung . . . bei einsetzender Nitratverarbeitung offenbar nicht ganz unbeeinflusst bleibt." This judgment does not quite agree with their own diagrams, from which the following figures can be drawn. At a moderate deficiency of nitrogen the gas exchange, with a respiratory quotient of around 1, amounted to about 38 units and after 4 hr to about 33; with the addition of nitrate the oxygen production after 4 hr had increased to 47 units, and the carbon dioxide consumption had decreased to 36. With a strong deficiency the corresponding figures for controls without nitrate were 28 and 30 units, respectively, and with nitrate added the oxygen production after 4 hr was 37 units, and the carbon dioxide consumption 24 units [all figures approximately estimated from diagrams (cf. Pirson, 1937)]. Myers (1949) likewise found a simultaneous increase in oxygen and a decrease in carbon dioxide at a high light intensity; without nitrate the oxygen/carbon dioxide ratio is 31.6/31.4, compared with an oxygen/carbon dioxide ratio of 39.0/28.7 in the presence of nitrate. It is difficult to escape the impression that, at saturating light intensities, nitrate mainly causes an increased oxygen evolution and a definitely less pronounced decrease in the carbon dioxide consumption. This would imply an increased utilization of light energy by the reduction of nitrate if we could disregard the possibility of a simultaneous decrease in the carbon dioxide consumption through the competition with nitrate and a general increase in the level of photosynthesis owing to the improved nitrogen status of the plant (cf. Pirson and Wilhelmi, 1950).

The case of Pirson and Wilhelmi with strong nitrogen deficiency and an obviously reduced carbon dioxide value ought to correspond to Myers's high-light plants with assumed high content of carbohydrates. Here the respiratory reduction of nitrate predominates and causes a decrease in the carbon dioxide intake.

More confusing are the low-carbohydrate plants in Myers's experiments. They should illustrate a condition with photochemical nitrate reduction with a limited supply of light. Thus there ought to be, just in this instance, a competition between nitrogen and carbon dioxide for light energy if such a phenomenon occurs and, consequently, a reduced carbon dioxide assimilation together with a constant photolytical formation of oxygen. On the contrary, the figures definitely indicate an increased formation of oxygen, which must be interpreted as a reduction of nitrate independent of the assimilation of carbon dioxide. It is thus impossible, from the gas-exchange data with *Chlorella*, to arrive at a clear picture of the relation between the assimilation of carbon dioxide and nitrate and the utilization of light energy.

In wheat plants the conditions are much simpler owing to the lack of dark reduction, and there is no doubt that the assimilation of carbon dioxide proceeds independently of the assimilation of nitrate, as shown in Table 8-2.

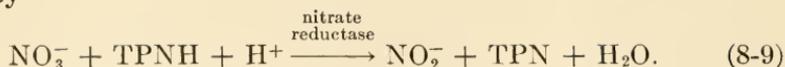
Thus there seem to be no experimental data necessitating the assumption of a competition between nitrate and carbon dioxide for a limited amount of available light energy, but several findings can most likely be interpreted as a nitrate reduction adding itself to that of carbon dioxide according to Eq. (8-7). There are, however, no real proofs of the strict validity of the equation.

Furthermore such a simple picture is perhaps chemically less plausible because the reduction of nitrate involves a successive reduction over intermediates. In green leaves no such intermediary inorganic compounds are definitely known to appear. It was therefore assumed (Burström, 1945) that especially the light-induced assimilation of nitrate involves an early fixation of nitrate in an organic linkage and subsequent reduction. That the assimilation of carbon dioxide is known to follow this pattern increases the probability of the picture. Another point of interest in this connection is that, in both *Chlorella* and wheat, organic nitrogen compounds seem to appear as direct assimilates and that they have a definite composition characteristic of the cells but independent of the light intensity. In nongrowing leaves they are of the approximate elementary composition of amino acids. This might imply that carbon dioxide and nitrate are reduced simultaneously in one system, leading directly to amino compounds. A fixation of nitrate or eventually some simple inorganic product of reduction to some intermediate in the carbon dioxide assimilation would afford a simple basis for such a synthesis.

The enzyme chemical studies of Evans and Nason (1953) have provided strong evidences of a photochemical reduction located in the grana fraction in leaves and based on the reaction



followed by



This important result fits in well with the conclusions drawn from the physiological experiments. It implies a photolytic system adding itself to that of the carbon dioxide assimilation, but it can account for only one-fourth the extra gas expected according to Eq. (8-7) or, in the case of a dark production of TPNH, Eq. (8-5), because it describes only the reduction of nitrate to the nitrite level. The fate of the nitrite nitrogen is still unknown, and so is the role of light in its reduction.

However, the results have led Evans and Nason to assume that the difference between the light and dark reactions may lie only in the mode of formation of TPNH. From the nitrite stage the assimilation of nitrogen could follow the same course. It must then obviously be postulated that the carbohydrate derivatives or reducing agents necessary for the following steps of the assimilation are either of the same origin in light and darkness—formed in the respiration—or that the principle of Evans and Nason is repeated. This means that the symbol  $X$  in Eq. (8-4), which stands for the whole sequence of reactants necessary for the complete reduction and assimilation of nitrate, can arise either in connection with the photosynthesis or in the respiratory metabolism. The picture outlined by Calvin and Benson (1948), to the effect that carbon dioxide assimilation is at least partly the reversal of the respiration with phosphoglyceric acid in the center, is interesting in this connection.

It is also worth mentioning that Kamen and Gest (1949) have demonstrated the presence of a light-stimulated nitrogen-fixing system in *Rhodospirillum* by means of  $\text{N}^{15}$ . The connection may seem remote, but there are, on the other hand, unmistakable similarities between the nitrogen fixation and the reduction of nitrate, for instance, in regard to hypothetical intermediates (Virtanen, 1950) and the dependence upon molybdenum, but the particulars of the processes are too little known to warrant more than mention without comments.

#### ADDENDUM

This manuscript was completed in 1951 and a few additions were made in 1953. The author regrets that he has not had an opportunity to pay regard to the important contributions by the following:

- Kessler, E. (1953) *Flora*, 140: 1-38, and *Arch. Mikrobiol.*, 19: 438-457.  
 Van Niel, C. B., M. B. Allen, and B. E. Wright (1953) *Biochim. et Biophys. Acta*, 12: 67-74.

They give answers to some of the main points discussed in this chapter and make this treatise, on the whole, obsolete.

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## CHAPTER 9

# Phototropism

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Phototropism is the response shown by plants when they bend within a light gradient. This response may be either a bending toward the light, which is positive phototropism, or a bending away from the light, which is negative phototropism. There are also plant organs, such as leaves, which place themselves at some definite angle to the incident rays. This kind of response is called "diaphototropism." Extensive reviews of phototropism can be found in du Buy and Nuernbergk (1932), Johnston (1936), van Overbeek (1939), and Galston (1950).

In most cases the phototropic reaction is a growth movement; consequently only the younger parts of the plant are involved. In a few cases, such as in the leaves of *Malva* (Yin, 1938), the movement is due to turgor change; this response can continue as long as the reacting tissue remains alive. Turgor movements do not always lead to phototropic but usually to photonastic responses (in which the direction of the movement is determined by the structure of the organ and not by the direction of the light), such as occur in most leguminous leaves. The mechanism of these movements has been investigated in detail by M. Brauner (1932) and L. Brauner (1948).

During the last part of the nineteenth century and the beginning of this century, a great deal of work was done on phototropism and its theoretical explanation. Since our knowledge of the phototropic response of the *Avena* coleoptile is rather complete as compared with what is known of other organs and species of plants, it seems logical to analyze this case in detail and then compare phototropic response in other plants with that found in *Avena*.

Growing *Avena* coleoptiles in weak red light for the first 30 hr after germination represses the growth of the mesocotyl or first internode. At that time the coleoptiles are strongly negatively geotropic and are growing straight upward. When they have a length of approximately 30 mm, they are highly sensitive to light; they respond within 1 hr, curving toward the light, when they have received as little as 1 m-c for

20 sec. As Darwin (1880) had already noticed, the zone of curvature does not have to be illuminated, since it is primarily the tip of the coleoptile which perceives the light stimulus. Therefore perception and reaction in phototropism are separated both in space and in time; the intervening processes of transmission of the stimulus account for this.

The quantitative aspects of phototropism were simultaneously elucidated by Blaauw (1909) and Fröschel (1908), both of whom found that minimal curvature is produced by a definite amount of light energy. Blaauw showed this amount of light to be about 20 m-c-sec, irrespective of the light intensity. This is shown in Table 9-1. Thus the product law of phototropism was established, which states that the response to

TABLE 9-1. THE RELATION BETWEEN LIGHT INTENSITY AND DURATION OF EXPOSURE NECESSARY TO GIVE A JUST-VISIBLE CURVATURE IN *Avena* COLEOPTILE (Blaauw, 1909.)

Length of exposure	Light intensity, m-c	Total light energy, m-c-sec
60 min.....	0.0048	17.2
15 min.....	0.025	22.4
4 min.....	0.090	21.6
8 sec.....	3.03	24.2
1 sec.....	18.9	18.9
0.04 sec.....	511	20.5
0.01 sec.....	1,902	19.0
0.001 sec.....	26,590	26.5

unilateral light is a function of the product of light intensity and duration of illumination. Further work, especially by Arisz (1915), showed that curvature was a periodic function of the light energy. Between 4 and 400 m-c-sec the phototropic curvature increases with increasing amount of illumination, and beyond that the curvature decreases with increasing light until a total light energy of 2000-4000 m-c-sec is reached. Between 2000-4000 and 20,000-40,000 m-c-sec negative curvatures may be produced. At still greater amounts of light a so-called "second positive curvature" occurs. Du Buy and Nuernbergk (1932) showed that at around 1,000,000 m-c-sec there was another region of light energy where the plants responded only slightly or not at all, but beyond that higher energies produced a third positive curvature.

In stimulation reactions it was found that the law of Weber-Fechner held. This law states that, when an organism is subjected to two stimuli of different intensity, the response is not due to the absolute difference in intensity but is proportional to the ratio between the two stimuli. Thus, when an *Avena* coleoptile is illuminated from opposite sides by

light of different intensities, curvature will occur only when one light source is 1.20 times as intense as the other (Pringsheim, 1926). To explain this, we have to admit that the sensitivity to light is reduced by light itself; thus the tonus, or *Stimmung*, of the coleoptile decreases as it is subjected to a greater amount of light. This was analyzed in detail by Arisz (1915).

Lange (1927) and Sierp and Seybold (1926) have measured the sensitivity of different regions of the coleoptile to light in numerous experiments. They exposed the extreme tip or narrow transverse bands of the coleoptile to unilateral light and determined the amount necessary to cause a just visible curvature. They found that the extreme 0.1 mm of the coleoptile tip was the most sensitive to light. From that point the sensitivity dropped about 5000 times to a rather constant value for all regions more than 2 mm distant from the tip. Therefore, when a coleoptile is illuminated with a sufficiently large amount of light, the basal cells will also respond directly to light. We should therefore distinguish between the tip response and the base response in the *Avena* coleoptile. These differ, first, in the amount of light necessary to produce a response and, secondly, in the location of perception of the light. There is transmission of the stimulus in the tip response but no such transmission in the base response, since the illuminated cells in the base respond directly. The third difference is significant in this connection: the base response occurs within half an hour after illumination, whereas it takes almost an hour before a curvature starts to develop as a result of the tip response. As a fourth difference might be mentioned the work of Haig (1935) in which a slight disparity in spectral sensitivity was found between the tip and the base response.

Between 1914 and 1918 Blaauw (1914, 1915, 1918) formulated his theory of phototropism, stating that a phototropic response results when local differences in growth rate are produced through local differences in light intensity. He based this theory on extensive series of measurements in which he showed that the growth of any phototropically sensitive organ is either retarded or accelerated by light. Therefore, when an organ is subjected to unilateral illumination of unequal intensity on either side, these local differences in light intensity produce local differences in growth rate, which in turn account for the phototropic curvature. This theory was hotly contested, and during the 1920's scores of papers appeared, either for or against Blaauw's theory.

Blaauw's theory does not account for a transmission of stimulus from tip to base. A new theory was formulated, almost simultaneously, by Cholodny (1927) and Went (1928a). This theory is based on the fact that the lower zones of the *Avena* grow under the influence of the growth-promoting substance auxin, which is formed in the coleoptile tip. The auxin is normally distributed equally over the different sides of the

coleoptile and therefore makes all sides grow at the same rate. The Cholodny-Went theory states that unilateral illumination causes a lateral shift in the downward-moving auxin, causing unequal distribution of auxin in the growing zones, which in turn causes unequal growth and curvature. The main difference between this and Blaauw's theory is that in one case the coleoptile reacts as a whole, because the front grows as much less as the back grows more, and in the other case each cell responds independently of the others. Another advantage of this theory is that it is applicable as well to geotropism, for which it has been proved by many investigators (Dolk, 1936). Without going into all the different experiments that purportedly differentiated between the two theories, let us first see whether an integration is possible.

Van Overbeek (1933) showed that the phototropic curvature in *Raphanus* hypocotyls could be explained quantitatively by a combination of Blaauw's theory and the auxin-redistribution principle. About 50 per cent of the phototropic curvature was due to the lateral transport of auxin, and the other 50 per cent was due to a change in the reactivity of the cells to auxin under the influence of light.

A number of experiments have shown that the auxin in *Avena* coleoptiles actually becomes redistributed under the influence of unilateral illumination. Went (1928a) placed coleoptile tips astride two agar blocks, separated by mica, and thus collected separately the auxin diffusing from the two halves of the tips. He found that, during the first 1.5 hr after unilateral illumination with 1000 m-c-sec, the total amount of auxin diffusing from the coleoptile tip amounted to 84 per cent of that diffusing from the tips of coleoptiles kept in darkness. But in the lighted coleoptiles 27 per cent of the total auxin (in darkness) was reaching the side facing the light, and 57 per cent arrived at the side away from the light. This means that the dark side received more auxin than it would have if the whole coleoptile had been kept in total darkness. In other experiments it was found that the difference in auxin diffusion from the light and dark sides increases during the second or third hour after illumination. In an experiment in which coleoptile tips were unilaterally illuminated with 100 m-c-sec, during the first 75 min 41 per cent of the auxin diffused from the light side and 59 per cent from the dark side. During the next 75-min period exactly the same amount of auxin diffused from the coleoptile tips, but only 11 per cent went to the light side and 89 per cent to the dark side. The data of Arisz (1915) show that this behavior of lateral auxin transport is in complete agreement with the rate of phototropic curvature. The rate of curving of the coleoptile reaches its maximum after 1.5 hr of illumination; from then on it continues to bend at the same rate for several hours. Therefore the main auxin differential induced by light should persist from the first to the fourth hour after illumination, particularly. This indicates the need for

more data on auxin redistribution an hour or more after illumination rather than immediately afterward.

Asana (1938) studied the auxin redistribution in *Avena* coleoptile tips that were subjected to 14,000 m-c-sec, an amount of light that usually produces a negative curvature. He established that the amount of diffusing auxin was the same in the illuminated tips as it was in the dark controls; therefore this amount of light does not alter the total auxin content. But at this high intensity 58 per cent of the auxin diffused from the light side, and 42 per cent came from the dark side. Asana's results were based on individual auxin diffusions from about 200 plants, and a statistical analysis showed that this difference was significant. The work of Wilden (1939) confirms both Went's and Asana's results. She determined the amount of auxin diffusing from either the illuminated or

TABLE 9-2. AUXIN DIFFUSING FROM ILLUMINATED AND DARKENED COLEOPTILE TIPS (Wilden, 1939.)

Curvature	Proportion of auxin diffusing from lighted side to that from dark side of coleoptile	
	During 1st hour after illumination	During 3rd hour after illumination
1st positive.....	42/58	17/83
1st negative.....	58/42	62/38
2nd positive.....	.....	36/64

the darkened side of coleoptile tips by placing them one-sidedly on *Avena* test plants. Table 9-2 shows her results.

Other investigators (Burkholder and Johnston, 1937) demonstrated a lateral transport of auxin in *Avena* coleoptile tips when the amounts of light that give the third positive curvature were used.

In later work Oppenorth (1941) studied the effect of light on the auxin content of the illuminated and shaded sides of *Avena* coleoptiles, using the ether-extraction method. Such extractions do not give direct proof of the lateral transport of auxin, but the evidence can be most readily explained by redistribution of auxin under the influence of light. Oppenorth, like Stewart and Went (1940), found that there is a certain amount of auxin destruction in light, but that this destruction is independent of the total amount of light applied. Therefore only a slight difference in extractable auxin content of the light and dark sides could be expected. It is clear that this auxin destruction cannot account for the large phototropic curvatures produced with small amounts of light.

Less direct but equally conclusive evidence of lateral auxin transport was given by Boysen-Jensen (1928). He split *Avena* coleoptile tips and

placed a thin slice of mica in the slit. When the light fell on the coleoptile parallel with the mica, normal phototropic curvatures would appear, but when the light source was perpendicular to the mica, there would be hardly any curvature at all. Since mica does not interfere with light, and consequently with auxin destruction, the only explanation that will fit the evidence is that the mica interferes with the lateral transport of auxin.

We can now apply these data to the phototropic response of the *Avena* coleoptile. When the coleoptile is illuminated with small amounts of light (under 1000 m-c-sec), only the tip is sensitive, and a curvature results after the differential auxin distribution induced by light has been transmitted from the tip to the growing zones. With strong illumination the lower zones also become sensitive, and then the Blaauw mechanism comes into action.

In Table 9-3 all facts pertaining to phototropic curvature of *Avena* coleoptiles are compared; it can be seen that a very clear-cut picture emerges. The Cholodny-Went theory explains the curvature resulting from low-intensity illumination; it is clearly based on phenomena occurring in the extreme tip of the coleoptile. The Blaauw theory explains at least part of the curvatures resulting from high-intensity illumination. Basing these principal differences on basic differences in light-perceiving processes is a very attractive proposition; this is actually supported by all the facts presented here.

Let us consider first the possibility of a differential destruction of auxin under the influence of light. Numerous investigators have shown that less auxin can be extracted after the coleoptiles have been illuminated than before. This has been attributed to the destruction of auxin by light. There are several mechanisms by which this destruction can take place. Kögl *et al.* (1936) have shown that both auxin-*a* and auxin-*b* are rapidly inactivated by ultraviolet light. Schuringa (1941) showed that this inactivation can occur in visible light when carotene is present. Galston and Baker (1949) showed that indoleacetic acid is readily destroyed by visible light in the presence of riboflavin. In view of the only minor destruction of the diffusible auxin by light and in consideration of the fact that the over-all growth of the coleoptile is only slightly decreased by illumination, it seems safe to conclude that neither auxin-*a* nor indoleacetic acid photoinactivation can play a prominent part in phototropism in *Avena*. Only curvatures of a few degrees could possibly be attributed to differential auxin destruction on both sides of the coleoptile. Therefore it is certain that the first positive curvature cannot possibly be due to photoinactivation of auxin. On the other hand, the weak second and especially third positive curvatures are of the proper magnitude. It would seem, then, that the base response of the *Avena* coleoptile can be satisfactorily explained by auxin destruction through

light absorbed by riboflavin. The total amount of auxin destroyed is small, on the order of 10-20 per cent, so that the differential growth retardation cannot be very great. The curvatures produced by illumination of the base never exceed a few degrees; therefore they can be explained by differential auxin destruction. The action spectrum

TABLE 9-3. PHOTOTROPIC AND GROWTH RESPONSES OF *Avena* COLEOPTILES

	Illumination of tip	Illumination of base
Minimal effective amount of light to cause phototropic curvature, m-c-sec. . . . .	20	50,000
Time lapse between illumination and starting of curvature. . . . .	About 1 hr	Less than 0.5 hr
Transmission of stimulus. . . . .	Transmission of stimulus from tip to reacting cells	Response local, where illuminated; some transmission downward
Spectral sensitivity. . . . .	Action spectrum resembles most the absorption spectrum of carotene	Action spectrum resembles most the absorption spectrum of riboflavin
Pigment concentration. . . . .	Highest concentration of carotene in tip	Riboflavin distributed evenly over whole coleoptile
Light-growth response after illumination	So-called "tip response," maximum growth retardation about 1 hr after illumination	So-called "base response," maximum growth retardation within half an hour after illumination
Auxin relations. . . . .	Auxin continuously produced in tip; can be collected by diffusion	Auxin present in extractable form and also supplied by tip, but very little obtainable by diffusion
Possibilities for photoinactivation of auxin	Auxin formed in tip and diffusing downward apparently not destroyed by light	Some inactivation of extractable auxin by light
Type of auxin. . . . .	Auxin- <i>a</i>	Indoleacetic acid
Possibilities for lateral transport of auxin	Solid tip; slight lateral displacement will cause complete redistribution lower down in coleoptile	Hollow cylinder; auxin has to move around periphery before an effective auxin gradient between dark and light sides is produced
Phototropic theory applicable	Cholodny-Went theory	Blaauw theory

obtained by Haig (1935) for the phototropic response of the base closely agrees with the riboflavin absorption spectrum.

When the actual growth of the tip-illuminated coleoptile is measured during the development of the phototropic curvature, it is found that the rear may double its growth, whereas the growth of the front comes to

almost a complete standstill (Went, 1928b; Beyer, 1928). This fact cannot possibly be explained by differential auxin destruction. In the first place, the growth along the rear is greater in light than in complete darkness. In the second place, not more than 20 per cent destruction of auxin has been reported, but still the growth of the front may cease altogether. However, these facts can be brought in line with the Cholodny-Went theory; a redistribution of auxin in the tip would increase the growth of the rear as much as it would decrease the growth of the front. Most investigators have not found a complete redistribution of auxin under the influence of unilateral light, but this is probably due to the fact that auxin redistributions were usually measured only during the first hour of illumination.

The facts presented thus far show a curious discrepancy when analyzed in view of the findings of Thimann and Bonner (1933) and Went (1942). These investigators found that extractable auxin in the lower zones of a decapitated *Avena* coleoptile decreased in 2 hr to approximately 50 per cent of that found in intact coleoptiles. This means that, if the extractable auxin were responsible for the growth of a coleoptile, the growth rate of a unilaterally illuminated coleoptile could never drop to less than half the normal rate within 2 hr after illumination. Since growth on the illuminated side may cease altogether, it is obvious that the extractable auxin is not responsible for growth. This is contrary to Went's conclusion (1942) but seems inescapable on the basis of the recently discussed facts. It also follows from Oppenorth's work (1941), in which a considerable decrease was found in ether-soluble auxin on the illuminated side of an *Avena* coleoptile, but enough was left to cause growth.

It seems possible to reconcile the opposing facts on the basis of the following hypotheses:

1. The auxin found in the coleoptile tip is auxin-*a*, as Kögl *et al.* (1934) have shown to be very likely. The evidence of Wildman and Bonner (1948), who showed that about 50 per cent of the auxin diffusing from the tip of the coleoptile is indoleacetic acid, is inconclusive on the basis of two facts: (a) The Salkowsky test is not specific for indoleacetic acid, as they claim, but gives a color with many other indole derivatives that are inactive in growth production (Gordon and Weber, 1951). Besides, the color produced from their coleoptile diffusates was not typical of the indoleacetic acid reaction. (b) A number of substances could have diffused out of the cut cells and have given positive results with the Salkowsky test. Also the diffusion-rate values obtained from coleoptile-tip diffusates indicate the presence of a substance with a much greater molecular weight than indoleacetic acid.

2. The auxin-*a* produced in the tip can be redistributed under the influence of light absorbed by carotenoids. The evidence of Haig (1935), Wald and du Buy (1936), and Bünning (1937) indicates carotenoids to be

the pigment that absorbs the light. Bandurski *et al.* (1950) have shown that albino corn seedlings, from which they were unable to extract measurable amounts of carotene, were as phototropically sensitive as ordinary corn seedlings that did contain carotene. These same experiments had been carried out by the present author in 1940 (unpublished). At that time it was found that not a single one of the white corn coleoptiles was without carotene. To demonstrate the presence of very minute amounts, the petroleum-ether extract of individual coleoptiles was sucked through a miniature chromatographic column made from a capillary tube filled with alumina, and in every case the yellow carotene band appeared. It was visible because the surface of the column was large compared with its diameter. It therefore seems doubtful whether real carotene-free coleoptile tips exist.

3. The auxin extractable from the lower parts of the coleoptile is almost certainly partially indoleacetic acid. The fact that growth may cease almost completely while considerable amounts of indoleacetic acid remain in the cells makes it likely that this generally distributed indoleacetic acid is responsible for at most only a small fraction of the growth of the coleoptile.

4. As Galston and Baker (1949) have shown, the indoleacetic acid is readily destroyed by light absorbed by riboflavin; this destruction may account for the relatively small curvatures produced by basal illumination.

There is a different explanation possible for the effects of light on the growth of the basal zones. Van Overbeek (1936) has shown that, when *Avena* coleoptiles are preilluminated, their subsequent response to indoleacetic acid is increased. This is obviously an indirect effect and may be due to a change in the sensitivity of the cells to auxin. Therefore, although the hypothesis of indoleacetic acid destruction on basal illumination of the coleoptile is very attractive, it is not necessarily the only possible explanation. Since it appears that such a small fraction of the extractable indoleacetic acid is destroyed, it would be very difficult to decide whether or not the destroyed fraction had any direct relation to growth.

Several investigations have been made to estimate the absorption of light within the illuminated coleoptile. The work of Nuernbergk (1927) is the most extensive. He concluded that the decrease in light intensity from front to back is rather uniform in the basal zones of the coleoptile. Here the enclosed primary leaf accounts for most of the light absorption. The light intensity on the rear is about 3 per cent of the incident light. In the extreme tip the light conditions were found to be more variable; no constant drop in light intensity was found.

For many years there was considerable discussion of whether the phototropic curvature was caused by the direction of light rays or by

the decrease in light intensity from front to back. Rather complicated experiments with unilateral illumination seemed to favor the light-direction hypothesis, but a simple experiment by Buder (1920) unambiguously proved the light-intensity hypothesis to be correct. He inserted into an isolated coleoptile a glass rod, the tip of which was bent at a right angle and which was silvered except at the ends. Light introduced through the base of this rod would thereby hit the inside surface of the coleoptile, and light direction and light diminution worked in opposite ways. The phototropic curvatures then obtained were always according to the decrease in light intensity.

Several suggestions have been made concerning the mechanism of the lateral transport of auxin under the influence of unilateral illumination. Went (1932) suggested that the potential difference between the illuminated and dark sides, which had been measured by Waller, was responsible for the lateral movement. Since the illuminated side of a plant part becomes negative in comparison with the dark side, it seemed possible that the anions of auxin could move within this potential gradient. The experiments of Clark (1937, 1938) did not support this theory, because he could not correlate the measurements of electrical polarity with the polarity of auxin transport. Schrank (1950) has shown in more recent work that phototropic curvatures are reduced in the same way as electrically induced curvatures by filling the inside of the coleoptile with an electric conductor, such as a nutrient solution. Introduction of non-conductors into the coleoptile did not change their sensitivity. Schrank also showed that an electrical potential could enhance or decrease a phototropic curvature, depending on whether it increased or decreased the electrical potential induced by unilateral light. Therefore his experiments tend to support the idea that the lateral auxin transport after phototropic stimulation is due to the induced electrical potential. Went (1936) calculated that approximately 0.001 erg is required for a lateral transport of auxin in the tip sufficient to give phototropic curvature. The minimal amount of light energy required for such a curvature is about 1 erg. This fact makes it possible to view the induction of phototropic curvature as normal physicochemical reaction, for which the energy is supplied by the exciting light.

There are some important experiments carried out by Koningsberger and Verkaaik (1938) which indicate that the base of the *Avena* coleoptile is sensitive to light only when a gradient of auxin-*a* exists from tip to base. When the bases of coleoptiles were illuminated from one side with 100 m-c, a phototropic curvature of 11° was obtained. Decapitated coleoptiles with pure agar blocks applied to their cut surfaces produced a curvature of only 1° or less. When agar blocks containing auxin-*a* were applied, curvatures of 7°-8° appeared, whereas decapitated plants with indoleacetic acid agar blocks produced curvatures of less than 1°. These

experiments do not agree with the suggestion that the base response is due to the destruction of indoleacetic acid by light. According to these experiments, both base and tip response can be due only to lateral transport or inactivation of auxin-*a*.

The previous analysis shows that most of the phototropic response of *Avena* coleoptiles is due to the lateral transport of auxin, particularly in the extreme tip. Van Overbeek showed that in *Raphanus* only 50 per cent of the curvature could be accounted for by lateral redistribution of auxin. Blaauw's calculations (1915) indicate that almost all the phototropic response of *Helianthus* hypocotyls can be explained by the differential response of the sides of the hypocotyl to light. When he calculated the amount of curvature produced with 512 m-c from the growth response of the light and shaded sides of the hypocotyl, he obtained a value of 11°. The actual curvature was 10°. Therefore it must be concluded that several different mechanisms exist in different plants, or coexist in one plant, which are all responsible for phototropic curvature. We might now try to summarize the various processes that are known or can be surmised to intervene between light absorption and phototropic response.

The action spectrum of phototropism, as determined by Blaauw (1909), Haig (1935), Johnston (1934), Bünning (1937), Galston and Baker (1949), and others, indicates that phototropically active light is absorbed by a pigment that has an absorption spectrum like those of the carotenes or riboflavin. As Galston (1950) has pointed out, an unequivocal decision between the activity of these pigments is difficult to reach. Yet the actual data agree best with the assumption that carotene-absorbed light is effective in inducing the tip reaction and the phototropic response of the coleoptile tip, whereas riboflavin-absorbed light is effective in causing phototropism in the base of the coleoptile.

From the auxin-extraction data and from the periodic nature of the degree of curvature in relation to light energy, we can say with certainty that auxin destruction plays at most a very minor role in the establishment of phototropic curvatures. Stewart and Went (1940), confirming du Buy's (1933) measurements, showed that the auxin diffusing through *Avena* coleoptiles is not destroyed by light at all. They found, as was confirmed by Oppenoorth (1941), that the ether-extractable auxin from *Avena* coleoptiles is reduced in amount by light, but that this decrease is not related to the amount of light energy and thus cannot be the basis for phototropic curvatures. It is very likely that this small amount of auxin destruction in light is responsible for the light-growth response of the base of the coleoptile, as measured by Dillewijn (1927) and Went (1941). It consists in a growth retardation, reaching its maximum extent after 25 min at 20°C, followed by a growth acceleration at 40 min. This response does not bear a quantitative relation to the amount of light

energy supplied; it is likely that this base light-growth response causes the phototropic oscillations described by Burkhardt (1926).

Whereas, at most, only a small amount of auxin *destruction* can be caused by the light that is absorbed, there are three other auxin effects that are produced by light. One is the effect of the unilateral light on the lateral transport of auxin. As just discussed, this may come about by a potential difference induced by the light, causing electrophoretic redistribution of auxin. If we assume that this potential is concentrated in the cell interfaces, its magnitude may be sufficient to account for the auxin movement. If this auxin, which is most likely auxin-*a*, is responsible for most of the actual cell elongation, its redistribution will demonstrate itself in a differential growth rate of the two sides of the coleoptile. The lag period between unilateral illumination and onset of curvature is slightly longer than the rate of auxin movement [about 15 mm/hr (Went and White, 1939)] would lead one to expect. This difference is probably due to the fact that the lateral auxin transport only gradually reaches a maximum value.

A third effect of light which has received theoretical consideration without much experimental support is that on auxin synthesis. Oppenoorth (1941) and Galston (1950) hypothesize that small amounts of light decrease auxin production in the *Avena* coleoptile tip, giving an increase at light energies causing negative curvatures and a decrease again at higher light-energy levels. The evidence for this is very meager and need not be considered at present.

A fourth effect of light has, surprisingly enough, hardly been considered: the reduced response of cells to auxin during and immediately after light exposure. Through extraction experiments we know that little or no auxin is destroyed by light; yet there is a considerable decrease in growth rate during and after illumination, as shown in the light-growth response, e.g., in *Helianthus* hypocotyls (Blaauw, 1915), *Raphanus* hypocotyls (van Overbeek, 1933), or in stems in general during the day. Therefore it must be concluded that cells respond less to auxin during and immediately after light exposure than they do in darkness. This may be due to blocking of the auxin or to inactivation of some other factors that, in conjunction with auxin, are needed to produce growth.

From all the facts presented it is clear that a unitarian theory of phototropism cannot account for all of them and therefore serves no practical end. As Table 9-2 shows, almost all the facts are consistent with a dual theory. It is very tempting to assume that the two completely different phototropic mechanisms are based on the effects of light on two different auxins. This would also tie in with the two pigment systems, each of which would be connected with a different auxin.

Of the extensive literature on phototropism in other plants, only a few

papers on light responses in fungi will be mentioned, because they have a direct bearing on the theory of phototropism.

Castle (1933) investigated the relation between light refraction and absorption and the direction of phototropic curvatures in great detail. Blaauw (1914) found that the growth of *Phycomyces* sporangiophores is accelerated by light. This seemed to contradict the explanation of the positive curvature produced by light; it means that the back is growing faster than the front. However, Blaauw had already pointed out that this could be explained by the lens action of the completely transparent sporangiophore, which focuses the light on the rear, thereby causing an acceleration of growth. Buder (1920) then tried immersing *Phycomyces* sporangiophores in paraffin oil and obtained negative curvatures. This, then, is a perfect proof that Blaauw's mechanism holds for phototropic curvatures in *Phycomyces*. The refinement in calculation made by Castle also confirmed Blaauw's views.

Van der Wey (1929) discovered a rather exceptional case of phototropic behavior in *Pilobolus*. The sporangiophores of this phycomycete shoot off their sporangia in the direction of light. The accuracy of this phototropic aiming can be measured by observing the spread of the sporangia that are shot against a glass plate fixed in front of a light source. When light hits the sporangiophores from two different directions, they aim at the arithmetical center between the light sources. When there is a 4° divergence between the light sources, half the sporangiophores aim at one, half at the other. From about 8° on, there is a complete separation in direction of curvature. Van der Wey tries to explain this by the assumption that the black sporangia intercept the light rays in such a fashion that no light reaches the ring of yellow pigment at the base of the sporangiophore swelling. Bünning (1937) has shown that light absorbed by this orange pigment is responsible for the phototropic response. Therefore any deviation of the sporangiophore from the direction of the incident light will cause the light to be absorbed and produce a growth response.

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## CHAPTER 10

# Photoperiodism

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*Introduction. Discovery. Photoperiodic responses of animals. Organ of perception. The period involved. Action spectra: Equivalence of phenomena—The time-measuring reaction. The pigment and the photoreaction. Reciprocity and energy response. Correlation of some responses to light. Photoperiodic aftereffects. Flowering in relation to auxins. Inheritance of responsiveness to photoperiod. Influence of environmental factors on photoperiodic response. Reviews on photoperiodism. Cognizance. References.*

### INTRODUCTION<sup>1</sup>

Biological responses indicative of reactions that serve in some way to measure duration of time are known for both plants and animals. These are in part associated with either the light or the dark period of the daily cycle and are subject to the seasonal variation of the night and the day lengths. Species adaptations of both plants and animals to change in season are often effected through this dependence. The term "photoperiodism" has been applied to one type of these responses associated with initiation of reproduction of plants and animals. Other responses are known to arise from the same initial reaction, and some of these are not necessarily periodic. Some periodic phenomena are of other classes, an example being those possibly determined by endogenous rhythms.

An attempt is made to treat a number of diversified biological responses that might in all species be associated with the same photoreaction or possibly with two different photoreactions, one in plants and the other in animals. There is danger that unrelated reactions have been brought into juxtaposition, but the risk is justified, if only as a challenge for more intensive study of the phenomena involved. The extensive literature, which has appeared in a thousand or more articles, is mentioned in the section Reviews on Photoperiodism. In general, attention in this chap-

<sup>1</sup> The names of organisms referred to in this chapter are those used by the authors of the literature cited. At the first reference to an organism its presently accepted name is included in parentheses.

ter is turned to that part of the work dealing with the initial reaction rather than to the details of the final biological expressions, although many of these are given as examples.

Perhaps one of the most striking peculiarities of photoperiodic responses is that they are often clearly shown by a particular variety of a species but are not apparent in closely related varieties or forms. This negative condition should be held in mind, since it too is a subtle part of the phenomenon indicative of a delicately poised system. It dictates careful consideration of the experimental conditions and reference to the forms studied.

### DISCOVERY

The clear-cut recognition of photoperiodism was due to Garner and Allard in 1920. They found that *Nicotiana tabacum* var. Maryland Mammoth was entirely vegetative during the normal summer days and those of early autumn in latitude 39° N. In the greenhouse, as the day further shortened and the night lengthened, the variety came into flower even when the plants were very young. Garner and Allard called plants of this type "short-day." They found that floral induction could be suppressed by shortening the dark period with light of low intensity.

Flowering of *Raphanus sativus* var. Scarlet Globe was promoted by long natural days, whereas its root enlargement took place on short days. Garner and Allard emphasized the flowering of this variety and called it "long-day." Climbing hempweed, *Mikania scandens*, which Allard (1938) studied later in greater detail, was found to be of an intermediate type, flowering most abundantly when the photoperiod was between 13.5 and 14.5 hr. Flowering of *N. tabacum* var. Connecticut Broadleaf, on the other hand, apparently did not depend upon day length. This variety was recognized as "indeterminate," or "day-neutral."

Garner and Allard in their first paper raised the question of photoperiodic control in animals, taking migration of birds as an illustration. Rowan (1926) was at that time observing the seasonal response of the finch, *Junco hyemalis*, and became convinced that photoperiodism was an important factor in the control of sexual reproduction. Marcovitch (1924) earlier had made extensive observations on control of reproduction in Aphididae and had come to similar conclusions.

During the same period Schaffner (1931) was concerned with the sexual irregularities that had been frequently observed in the flowering of hemp, *Cannabis sativa*. Hemp grown when the days are long and the nights short is normally fully dioecious, but on shorter days and longer nights male flowers frequently occur on female plants and a few female flowers sometimes appear on male plants.

Seasonal changes in reproduction are lore for animals. The common opinion seems to have been, perhaps quite naturally, that they were

determined by temperature. In 1932 Baker and Ranson, however, reported that reproduction in the field mouse, *Microtus agrestis*, almost ceased when the day length was reduced from 15 to 9 hr, the nights changing from short to long. At that time Bissonnette (1932a) reported work on reproduction of the ferret, *Putorius vulgaris* (*Mustela fure*). He found that the female could be brought into full oestrus by lengthening the period of light during winter. In both cases light of low intensity was adequate for induction of oestrus when used to extend the normal day. These are "long-day" animals, whereas others such as man appear to be indeterminate (Whitaker, 1938); however, note the comments of Tennyson (1842) and Cook (1894).

### PHOTOPERIODIC RESPONSES OF ANIMALS

Descriptive surveys of photoperiodic responses were made on many plant varieties in the two decades following the period of discovery. In the latter part of this period and more recently, attention turned to physiological aspects of the subject. Results of the physiological work on plants are discussed in detail and serve to illustrate the occurrence of the phenomenon. Photoperiodic responses of animals have not been studied as extensively as those of plants, and less is known about the physiological aspects. For this reason a brief presentation of descriptive aspects of the work with animals is given as a background.

Sexual reproduction in many types of animals is definitely regulated by photoperiodism. Just as there are long-day, short-day, and day-neutral plants, there are similar groups of animals. Some aphids are examples of short-day types. Marcovitch (1924) found that the production of the sexual forms, which occurs in late summer or fall, is conditioned by photoperiodism rather than other environmental factors such as temperature. Deer, sheep, and goats are other examples of short-day animals. In these the normal breeding season occurs during the short days of autumn, and the young are born in the spring (Bissonnette, 1936b, 1941; Simms, 1950; Yeates, 1949). Hafez (1950) found that the duration of the breeding season of sheep is related to their origin, breeds from the Scottish Highlands having a considerably shorter season than those from southern England, and the latter, a shorter season than breeds of Spanish origin.

Long-day animals include a great number of birds. Bissonnette (1936b) listed junco, crow, canary, starling, mejiro, turkey, chicken, sparrow, mourning dove, duck, pheasant, quail, and grouse. Long-day mammals include ferrets (Bissonnette, 1932a), field mice (Baker and Ranson, 1932), raccoons (Bissonnette and Csech, 1937), and many others. Ground squirrels, guinea pigs, and a fish, the stickleback, are reported to be uninfluenced by photoperiodism (Bissonnette, 1936b). Rowan (1938)

cited other work indicating that the stickleback is photoperiodically responsive, as is also reported for the brook trout.

Responses other than those concerned with sexual reproduction occur in animals as they do in plants. Typical of these is the production of winged forms of certain aphids, shown by Marcovitch (1924) to be regulated by the relative length of the night and the day. Different species of aphids present many variations, but in general they pass the winter in northern latitudes in the egg stage. The eggs hatch in the spring, and a wingless stem mother that reproduces parthenogenetically develops. In some species a winged migrant form appears during the late summer, and upon approach of autumn the winged forms produce males and females. Marcovitch observed that sexual forms of *Aphis forbesi*, *A. rumicis*, *A. sorbi*, and *Capitophorus hippophaes* appear only on short days. He found migrant forms to be either long-day-dependent, as *C. hippophaes*, or short-day-dependent, as the apple aphid, *A. sorbi*. Shull (1927) found that many more winged individuals of the potato aphid, *Macrosiphum solanifolii*, formed if they were reared in 24-hr cycles of 8 hr of light and 16 hr of dark than if they were grown in continuous light. Shull and Marcovitch both concluded that the action of light is a direct one on the aphid rather than an indirect one received through the host plant. Shull also found that photoperiod treatments are effective in control of wing production only when applied some hours before the aphid is born.

Diapause, a physiological state of arrested development which occurs at some stage in the life cycle of many insects, is photoperiodically controlled in some instances. Dickson (1949) found that the oriental fruit moth, *Grapholitha molesta*, did not enter diapause if held in continuous darkness or continuous light. When the larvae were subjected to 3–13 hr of light per day, the number entering diapause increased from about 10 per cent at 3 hr to nearly 100 per cent on the longer light period. With 14 hr of light per day, however, the percentage dropped abruptly to 4, and with further increase in day length it became practically zero, the response being similar to that of intermediate plants. Dickson's results indicate that the length of the dark period is a critical factor in determining whether the organism enters diapause. The response is complicated by interaction with temperature and possibly other factors.

Other photoperiodic responses of animals include the seasonal changes in plumage of birds and in hair types of certain mammals. Lyman (1943) made an intensive study of coat changes in the varying hare, *Lepus americanus*. This animal undergoes three molts, one in the spring and two in the fall. In the spring the white winter coat is replaced by a brown one. In late summer this brown coat is replaced by another brown one of a different type, and soon thereafter the second brown one is followed by the white winter coat. Each of these molts is photo-

periodically controlled, but the particular light requirement for each one is different. Preceding the molts, sometimes by as much as 2 months, physiological changes occur in the skin, and these determine the hair type and color at the next molt. The physiological condition of the skin at a certain time is determined by plucking a small area on the animal. New hair covers the plucked area, and its color is determined by the physiological stage reached by the skin at the time of plucking. During the first autumn molt, or very shortly thereafter, the skin changes to the physiologically white condition, and many weeks before the spring molt it again shifts from physiologically white to brown. Lyman found that artificially lengthened photoperiods in autumn prevented the change of skin condition from brown to white, or if the change had already occurred, the long-day treatment resulted in its reversal to the brown condition.

Although photoperiodism controls sexual reproduction in a great number of animals, the first evidence of its effects varies. In certain aphids (Märkovitch, 1924) the proper treatment results in production of sexual individuals instead of asexual ones. Certain birds and mammals respond to favorable treatment by a rapid increase in size of the gonads and by changes in their structure leading to the production of gametes. The first obvious effect in the snail (Jenner, 1951) is to promote ovulation. The gonad apparently contains fully formed eggs at all times, once the snail has attained a certain size, and the function of light is to cause their release. Proper day length results in earlier implantation of the embryo in some animals, such as the martin (Pearson and Enders, 1944), and thus in reduction of the period of gestation by several weeks. Despite these apparent differences in response of widely different species, it is not unlikely that the same initial light action is responsible for each.

#### ORGAN OF PERCEPTION

In plants the leaf is effective in the photoreaction controlling flowering and other morphogenic responses (Cajlachjan, 1936; Knott, 1934; Moskov, 1936). Dark periods favorable to flowering are effective when applied to a single leaf or part of a leaf of certain short-day plants, e.g., *Soja max* var. Biloxi (*Glycine max*) (Borthwick and Parker, 1938), *Xanthium pensylvanicum* (Hamner and Bonner, 1938), and *Kalanchoe blossfeldiana* (Harder, 1944, 1948). A leaf is most effective as soon as it is fully expanded. Presence of other leaves subject to light conditions noninductive for flowering apparently is not inhibitory except as might result from their effect on translocation. It does not follow that the stem and other parts are not also effective, particularly if the leaf is vestigial, as in *Asparagus officinalis* and species of cacti. Direct irradiation or darkening of the terminal meristem does not control floral initiation

(Borthwick and Parker, 1938; Knott, 1934; Withrow *et al.*, 1943) and tuber formation (Hamner and Long, 1939).

Many studies of the part played by translocation of the stimulus in the final expression of photoperiodic control have been reviewed by Murneek (1948) and Chlodny (1939). These studies often made use of branched or grafted plants, one part of which was subjected to photoperiodic conditions favorable for flowering, whereas the other was under unfavorable conditions. In these cases defoliation of the part under unfavorable photoperiods, probably by inducing translocation to it, promoted flowering. Translocation of the photoperiodic stimulus depends on contact of living cells (Withrow and Withrow, 1943) and seems to occur mainly in vascular tissue, as indicated by the work of Harder (1948) on *Kalanchoe*. Severing a petiole of an effective leaf and separating the parts by lens paper block the response (Withrow and Withrow, 1943).

Photoperiodic control of reproduction in some mammals and birds is associated with the eye and is mediated by the pituitary. If the eyes are covered, photoperiodic conditions that are otherwise satisfactory for reproduction produce no effect (Burger, 1949). Experiments of this type, however, do not prove that the eye is the effective organ. Benoit and Ott (1944) found in ducks covered except for the eyes that gonads developed in response to supplementary light. Sexual response of ducks to visible radiation after extirpation of the eyes and severing of the optic nerve convinced Benoit and Ott that the pituitary was directly responsive. Light-transmission tests on heads of ducks indicated that some visible radiation probably penetrated to the pituitary through the region of the eye. Bissonnette (1938), working with the ferret, considered that the eye was effective in perception. One ferret with cataracts was not responsive to light conditions adequate for production of oestrus in normal females, and cutting the optic nerves of others delayed response.

#### THE PERIOD INVOLVED

Photoperiodic responses are determined by the length of the dark period. The literature, particularly that before 1945 on plants and even that current for animals, turns attention almost entirely to the light period. Most of the experimental work makes use of 24-hr cycles, as is natural for experimental convenience, and thus establishes the length of both the light and the dark period. Garner and Allard (1920) in their first paper demonstrated that *Soja max* var. Peking and *Aster linariifolius* are short-day plants. They found, however, that darkening the plants for 4 hr in the middle of the light period was without effect. They stated in 1931: "Indeed the effect of midday darkening is much the same as if the plants remained in the light for the whole day."

Shull (1929), working with the potato aphid, *Macrosiphum solanifolii*, established that a cycle of 6 hr of light and 10 hr of darkness led to the same effect as a cycle of 14 hr of light and 10 hr of darkness. Under these conditions and at temperatures below 20°C, wingless forms produced wingless offspring, but on cycles of 12 hr of light and 12 hr of darkness almost all the progenies were winged. Thus dark periods shorter than 12 hr, rather than long light periods, were required for parthenogenetic reproduction as wingless forms.

Effectiveness of the dark period is best shown by its interruption with short light periods. The short-day plant Biloxi soybean, grown with 16-hr light periods, develops vegetatively but flowers when given cycles of 8 hr of light and 16 hr of darkness. If the 16-hr dark periods are interrupted after 8 hr for as short a time as 1 min with 160 ft-c of illumination, the plants remain vegetative (Emsweller *et al.*, 1941). In the case of *Xanthium pensylvanicum*, flowering is induced on a 9-hr dark period and a 15-hr light period, but if light of moderate intensity is given for 1 min at the middle of the dark period, floral primordia do not develop (Hamner and Bonner, 1938). Razumov (1941), working both with short-day plants such as *Perilla*, *Chrysanthemum*, and various kinds of millet and with long-day plants such as *Avena sativa*, found that the effectiveness of the dark period was nullified by a short interruption.

Hamner and Bonner (1938) found that a single long dark period leads to floral induction of *X. pensylvanicum*, but that a single short light period is without effect. In this species the dark period has to be greater than 8.5 hr for flower formation irrespective of the length of the light period. Thus plants on 16-hr light periods and 8-hr dark periods remain vegetative, whereas those on 16-hr light periods and 32-hr dark periods flower.

Interruption of dark periods by short periods of light has also been shown to be effective in controlling the photoperiodic response of some animals. Thus Jenner (1951) found that a snail, *Lymnaea palustris*, reacted as a long-day animal, producing eggs on 13.5-hr light and 10.5-hr dark periods but failing to do so on 11-hr light and 13-hr dark periods. It produced eggs freely on 9-hr photoperiods when the accompanying 15-hr dark periods were interrupted near the middle for 1 hr with 60 ft-c of light. D. S. Hart found, according to Yeates (1949), that ferrets subjected to interruption of long dark periods went into oestrus, again emphasizing the importance of the dark period.

Intermediate plants such as *Mikania scandens* (Allard, 1938), and *Saccharum spontaneum* (Sartoris, 1939) apparently require a dark period of very precise duration. Parthenogenetic production of summer migrant aphids by wingless forms might also be a response to dark periods of intermediate duration.

## ACTION SPECTRA

## EQUIVALENCE OF PHENOMENA

The incident energy or quanta in different wave-length regions required to reduce the effectiveness of a dark period otherwise adequate for a given physiological expression to some given level can be measured. These measurements in the case of photoperiodism have not been expressed in terms of energy absorbed by the pigment in the effective reaction. They are of value, nevertheless, as a first guide to the equivalence of the initial

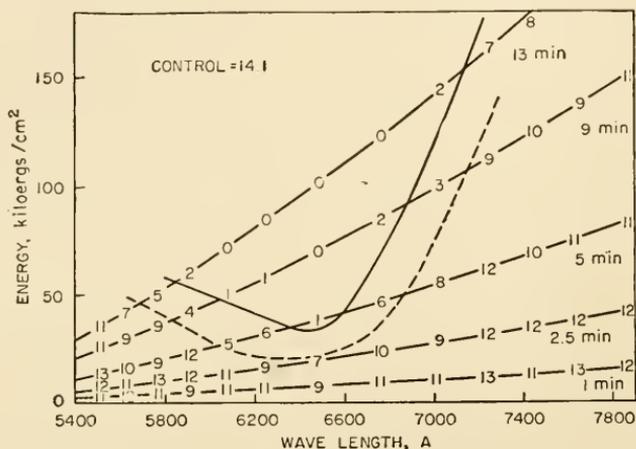


FIG. 10-1. Response curve for suppression of floral initiation in Biloxi soybean in the red region of the spectrum. Resulting energy at each wave-length station is plotted, and superimposed on energy lines are total numbers of flower primordia formed by lots of four plants which received a specific wave-length band at different energy levels. The curves were drawn freehand through energy lines, crossing between points at which sharp breaks occurred in the number of flower primordia produced. The solid curve was taken as the one representing the response, and the broken-line curve illustrates gradation effect with decreasing energy.

reaction in the several responses of plants. Quantitative information is restricted to plants, and even qualitative results are very limited for animals.

Such direct results, however, require correction for absorption by inactive pigments and scattering by tissue. This action spectrum gives the reciprocal of the relative absorption spectrum of the effective pigment, provided that (1) the total absorption is small, (2) absorbed quanta are equally effective independent of wave length, and (3) the light path is nearly the same at all wave lengths. The first and third requirements have been met in some cases for plants and could be met with some small animals such as mosquito larvae and aphids.

In plants the action spectrum can be found by irradiation of the leaf, followed by measurement of responses (Parker *et al.*, 1946). It is very convenient in this work to make use of dark-period interruption on a

single leaf, a single leaflet, or a whole plant. In this way duration of treatment with radiation of known intensity in a given wave-length band can be much shorter than required for extension of the photoperiod. Where the response is control of floral initiation, the plants are irradiated near the middle of several successive long nights. They can be returned to noninductive photoperiods for development prior to dissection.

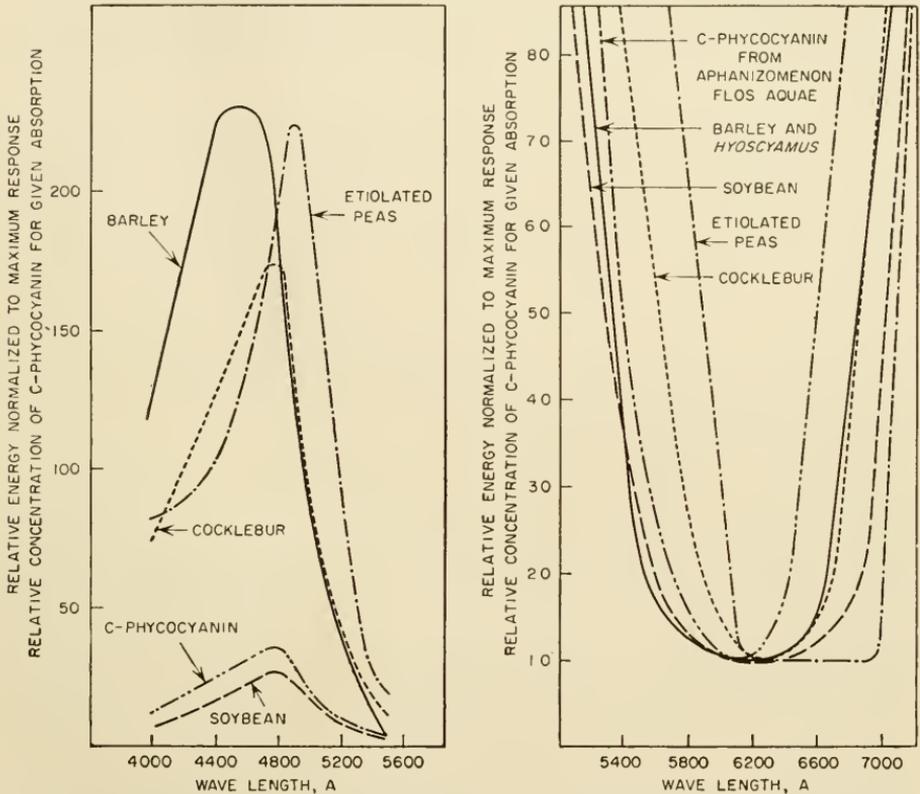


FIG. 10-2. Composite action curves for control of floral initiation of soybean, cocklebur, barley, and *Hyoscyamus* and for regulation of leaf size in etiolated peas, together with a curve showing the relative concentrations of C-phycoerythrin for a given absorption.

The wave-length bands used in measurement of the action spectra must have a very low level of extraneous radiation, since a given response for different wave-length regions might vary a thousandfold in its energy requirement. In the design of the radiation source a balance must be effected between area irradiated, wave-length regions isolated, and energy per square centimeter per second. A spectroscopist with two large prisms (*ibid.*) was used in obtaining the action spectra shown in Figs. 10-1 and 2. Results for floral initiation in *Soja max* var. Biloxi for the red portion of the spectrum are shown in Fig. 10-1 and throughout the region of effectiveness at wave lengths longer than 4000 Å in Fig. 10-2, above. The

energy values on the ordinate of Fig. 10-1 are given in absolute units, but relative values are shown in Fig. 10-2 since the choice of response level is arbitrary. Relative responses for some other plants are also shown in Fig. 10-2. Cocklebur, *Xanthium saccharatum*,<sup>2</sup> is similar to the soybean in requiring an adequately long dark period for flowering. Its floral-initiation response in the region of maximum effectiveness in the red is very similar to that of the soybean on an absolute energy scale. In both instances a total energy of about  $50 \times 10^3$  ergs/cm<sup>2</sup> in the region near 6500 Å, which can readily be given in less than 1 sec, is adequate to prevent floral induction.

Barley, *Hordeum vulgare* var. Wintex, and an inbred annual form of *Hyoscyamus niger* are representative of plants in which flowering is inhibited by an adequate dark period. This form of *Hyoscyamus* does not flower in response to 10 successive dark periods of 12 hr each. Interruption of long dark periods in a series of alternating light and dark periods causes *Hyoscyamus* and Wintex barley to flower. This is opposite to the response of soybean and cocklebur on interrupting the dark period. The long- and short-dark-period types of plants, nevertheless, have closely similar action spectra for floral initiation (Borthwick *et al.*, 1951; Parker, Hendricks, and Borthwick, 1950).

Qualitative results for formation of visible buds and open flowers of several plants have been obtained by a number of workers using wave-length bands separated by means of filters. The filters used were apparently adequate, but the energy required for a given response was not determined. Instead the response for a single energy level in several wave-length regions has often been measured. Such results cannot be completely evaluated unless the response is also measured as a function of energy. A relatively ineffective wave length can appear as effective as the most active region if the biological response approaches complete expression.

Razumov (1933) and Withrow and coworkers (Withrow and Benedict, 1936; Withrow and Biebel, 1936; Withrow and Withrow, 1940) extended the natural photoperiod with radiation obtained by use of filters. They reported that red radiation very effectively inhibited the flowering of short-day plants and promoted the flowering of long-day plants. Blue radiation was ineffective except for China aster, *Callistephus chinensis* var. Heart of France, which Withrow and Benedict (1936) found responsive to all wave lengths in the visible part of the spectrum at the energy level used. Kleshnin (1943, 1946), using *Perilla ocymoides* (*P. frutescens*) and oats var. Pobeda, also found that all parts of the visible spectrum were effective provided that sufficient energy was applied. Katunskij (1937), working with millet, hemp, green beans, and peas, observed that

<sup>2</sup> Plants of *X. saccharatum* (Parker *et al.*, 1946) were from a seed source used by Hamner and Bonner (1938) and referred to by them as *X. pensylvanicum*.

radiation in the blue and red portions of the spectrum was effective in controlling flowering, whereas radiation in the green was ineffective.

Funke (1936 through 1948) used radiation that had been passed through appropriate filters to isolate various wave-length regions to extend photoperiods of natural light. The energy levels were unequal, the red region probably having the highest value next to the unfiltered radiation. Funke measured his results by date of flowering and on this basis found four classes of plants, including examples of both long- and short-day types. Some examples from each of the four classes follow:

1. Red and unfiltered effective, blue ineffective, e.g., *Anthemis tinctoria*, *Lycopus europaeus*, *Solidago virgaurea*, *Cosmos bipinnatus*.

2. Red, unfiltered, and blue effective, e.g., *Mentha rotundifolia*, *Rudbeckia speciosa*, *Perilla nankinensis* (*P. frutescens* var. *Crispa*).

3. Unfiltered effective, red and blue ineffective, e.g., *Lepidium draba* (*Cardaria draba*).

4. Unfiltered and blue effective, red ineffective, e.g., *Iberis amara*, *Sinapis alba* (*Brassica hirta*).

The responses in the first three classes can be explained by action spectra of the type shown in Fig. 10-2, but this is not the case for the fourth class. Funke (1948) observed that all species belonging to the fourth class are Cruciferae.

Wassink *et al.* (1950) examined the response of *Brassica rapa*, a species of the fourth class, to supplementary irradiation in various wave-length regions. Their findings fully support those of Funke in showing a strong photoperiodic response to blue, violet, and infrared radiations, particularly for floral development and stem elongation. Green, yellow, and red radiations are essentially ineffective. In this work all plants were given 10-hr photoperiods at a constant energy level of about  $22 \times 10^3$  ergs/cm<sup>2</sup>/sec, with supplementary radiation of various colors for 8 hr at an energy level of  $1 \times 10^3$  or  $3 \times 10^3$  ergs/cm<sup>2</sup>/sec.

Photostimulation of the pituitary and the accompanying testicular growth of Peking ducks were studied by Benoit and Ott (1944). They used equal incident energies in various wave-length bands isolated by filters. Each duck was maintained in very dim blue light and was irradiated every third day for 8 or 15 hr alternately for 10 periods. Response was measured by the change in surface area of a testis exposed by laparotomy. They found the wave-length regions centering at about 6200 and 6800 Å to be most effective, with effectiveness gradually decreasing until 4400 Å, where the response was the same as that of controls. Wave lengths longer than 7000 Å were ineffective. When the radiation was reflected down quartz rods or tubes placed in the ocular cavity with the ends resting on thin bones above the pituitary, red and blue were approximately equally effective in bringing about enlargement of the testes. In these last experiments each duck was irradiated for 2 or 3 hr daily.

Bissonnette (1932b) and Burger (1943), working with the starling, *Sturnus vulgaris*, found red radiation to be most effective in stimulating sexual activity, with green less active and blue ineffective when used to extend a normal light period during winter. Similar results were obtained with turkey, *Melagris gallopavo*, by Scott and Payne (1937) and with the sparrow, *Passer domesticus*, by Ringoen (1942).

Oestrus in the female ferret was found by Marshall and Bowden (1934) to be uninfluenced by radiation beyond 7500 Å. Marked response was obtained throughout the visible spectrum at sufficiently high intensity. Barbanti (1932), working with the field mouse, *Microtus agrestis*, found that red and yellow radiation stimulated sexual activity.

Lengthening of certain internodes of various plants is inhibited by light. In this way the length of underground parts of most seedlings is regulated by light reaching the plants as they emerge. A single low-intensity irradiation for a short period can greatly affect the development of dark-grown seedlings. This, of course, is not a periodic phenomenon. Abnormalities that occur when plant growth takes place in darkness or at low light intensities are often grouped under the general term "etiolation." There is an extensive literature on etiolation, of which only one aspect, namely, elongation of certain organs of dark-grown seedlings, is considered.

An action spectrum for one type of etiolation, namely, that for leaf size of dark-grown pea, *Pisum sativum*, seedlings, has been obtained (Parker *et al.*, 1949). The results are shown in Fig. 10-2. Although these lack precision in the region of minimum effectiveness near 4600 Å, nevertheless they are closely related to the action spectra for control of flowering. Similar results were obtained for suppression of elongation in the second internode of barley, *Hordeum vulgare* (Borthwick *et al.*, 1951). Albino and potentially green plants of barley reacted alike. Again it is to be noted that the two action spectra dealing with elongation are equivalent even though the one enhances and the other inhibits lengthening of particular structures.

Goodwin and Owens (1948, 1951), using filters with mercury and sodium arcs, also found the red portion of the spectrum to be most effective for internode inhibition of oats, *Avena sativa* var. Victory. They measured the variation of response with energy incident on the complete seedling and determined the relative energies at various wave lengths required for a given inhibition of dark-grown seedlings. The action spectra closely follow those of Fig. 10-2, but some minor variations were interpreted as evidence of fine structure. These more likely arise from biological variation, as can be seen by inspection of Goodwin and Owens's curves showing variation of response with energy, particularly the curve for 5890 Å.

## THE TIME-MEASURING REACTION

Discoveries based on action spectra for seed germination (Borthwick, Hendricks, Parker, *et al.*, 1952) have clarified the nature of the photo-reactions and the time-measuring dark reaction in photoperiodism and related phenomena (Borthwick, Hendricks, and Parker, 1952). The characteristics of these action spectra are given in the discussion on seed germination by Evenari (Chap. 11). In brief, imbibed seed of *Lactuca sativa* var. Grand Rapids require visible radiation for germination with a minimum energy for response near 6600 Å. Germination is suppressed by radiation in the region 7000–8000 Å (Borthwick, Hendricks, Parker, *et al.*, 1952; Flint and McAlister, 1935), with a minimum energy requirement near 7350 Å (Borthwick, Hendricks, Parker, *et al.*, 1952). Some other lettuce varieties, as well as seed of *Amaranthus caudatus* (Resüher, 1939), *Phacelia tanacetifolia* (*ibid.*), and some varieties of tobacco, are similar in this response. The action spectrum for promotion of germination of these seed is closely similar to that described in the previous section for photoperiodism of soybean, cocklebur, and other plants.

The promotion and suppression of seed germination are controlled by a reversible photoreaction in which the pigment with maximum absorption near 6600 Å is changed by radiation in this region to an isomeric form with maximum absorption near 7350 Å. The latter form is returned to the former by irradiation in the region 7000–8000 Å. The interconversion can be made repeatedly (Borthwick, Hendricks, Parker, *et al.*, 1952).

These findings led to a search for a promotive action of radiation on floral initiation in the cocklebur (Borthwick, Hendricks, and Parker, 1952), in addition to the inhibitory one described in the preceding section. Such an effect was found for radiation in the near infrared, with a minimum energy required for response in the region of 7350 Å. The equivalence of the seed-germination action spectrum with that for photoperiodic response is thus clearly established. This equivalence permits the use of seed germination, which has many experimental merits, for further examination of the phenomenon.

That the pigment with an absorption maximum at 7350 Å changes thermally to the pigment with absorption maximum at 6600 Å is shown by the behavior of imbibed lettuce seed held several days in darkness at temperatures of 30°C or higher. Such seed fails to germinate upon return in darkness to lower temperatures irrespective of whether or not it was irradiated in the region 6000–7000 Å at the start of the treatment. If the seed is irradiated in this region at the end of the period at high temperature, it germinates at the lower temperature. Thus, although germination is blocked by the higher temperature, the pigment in the form to

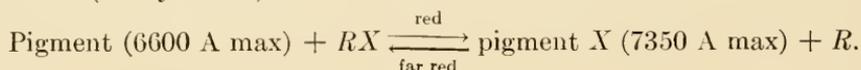
promote germination changes in darkness to the other form (*ibid.*; Borthwick, Hendricks, Parker, *et al.*, 1952).

That the change of the pigment in darkness is the time-controlling reaction in photoperiodism is shown not only by the equivalence of the action spectra but also by the effect on the critical night length of irradiation in the region of 7350 Å. *Xanthium pensylvanicum* in one experiment, for instance (Borthwick, Hendricks, and Parker, 1952), required a critical dark period of 8.5 hr for floral initiation. This was shortened to less than 6.0 hr by irradiation in the region of 7350 Å for 30 min just before the start of the dark period and lengthened to 9.0 hr by irradiation in the region of 6600 Å.

In short, during the day radiation maintains the pigment in the infra-red-absorbing form, and at night it slowly returns to the red-absorbing form. The reaction, although not yet tested on short-night plants, probably goes on in the same way in both long- and short-night plants. The opposite response in flowering of these plants is determined by some step later than the pigment reaction.

#### THE PIGMENT AND THE PHOTOREACTION

A simple form for the photoreaction and the time-controlled dark reaction (heavy arrow) is



This indicates that the photochemical change involves a reaction of the pigment with another molecule. It is observed that the rate of the photoreaction is independent of temperature, which requires the pigment to be in continuous collision with the other molecule. A first-order rate should be followed but the way in which this or any functional relation can be tested on a population is under debate at this time (Eddy, 1953). It is possible that the reversible photoreaction involves only the isomerization of a molecule, but this seems unlikely since any change in sensitivity to red radiation is accompanied by a change in sensitivity in the opposite direction to far-red radiation.

There are model reactions of the expected type, thus methylene blue can be reversibly photooxidized and reduced in the presence of ascorbic acid or iron salts.

These characteristics of the pigment, ascertained from response to red and infrared radiation, are adequate to facilitate its isolation. The bearing of further physiological observations on the nature of the pigment is of some interest, however, even though an unimportant alternative cannot be decided except by isolation.

Biologically active pigments of the types now known to absorb radi-

ation by electronic transitions in the far red or near infrared have of the order of 20 or more conjugate double bonds (Strain, 1949). The most likely ones for consideration here are cyclic and open-chain tetrapyrroles. The positions of the red and infrared maxima for physiological action are in agreement with either type of pigment, and the distinction between the two classes must depend on the absorption in the region 4000–5000 Å; the cyclic tetrapyrroles such as the porphyrins have a high absorption in this region owing to the so-called "Verdet bands" (Rabinowitch, 1944), and the open-chain tetrapyrroles such as the prosthetic group of phyco-cyanin (Svedberg and Katsurai, 1929) have low absorptions.

The action spectra for photoperiodism and for seed germination show relatively low physiological response in the blue compared with the red and infrared parts of the spectrum. From seed germination it is apparent that the absorptions of the two pigment forms overlap in the region below 5000 Å. The details of this response, however, show that the approach to photoequilibrium between the two forms requires an irradiance at least tenfold greater than that near 7000 Å, where the infrared and red absorptions overlap and after appropriate times of imbibition prior to irradiation lead to the same germination as that effected in the blue region.

Low physiological response, however, might accompany a high absorption if the quantum efficiency for chemical change were low. The alternatives then are that (1) the pigment is an open-chain tetrapyrrole or (2) the pigment is a cyclic tetrapyrrole with low effectiveness of the Verdet bands for physiological action.

#### RECIPROCITY AND ENERGY RESPONSE

Information about the photoreactions and the biological response can be obtained from reciprocity experiments in which the variation in response with intensity of radiation is measured for constant energy. Further information is given by variation in response with change in energy.

Reciprocity holds in simple photoreactions from short times limited by rate of energy supply to times of the order of the half-life for back-reaction if a product of the system reverts in darkness and is not limited by a nonphotosensitive reactant. If a biological response is involved, reciprocity will hold only over a period in which biological change, such as growth, is small. Thus reciprocity might be expected to hold in the photoperiodic systems from fractions of a second to an hour or more, as has been observed for control of floral initiation of soybean and cocklebur (Parker *et al.*, 1946) and for leaf lengthening in pea (Parker *et al.*, 1949).

In seed germination, reciprocity is limited by the rate of change of sensitivity of the seed to radiation and by the rate of germination. Deviations from reciprocity for germination of light-sensitive lettuce seed and

for control of flowering can be noted for irradiation times greater than 2 hr in contrast to those less than 1 hr. The rates of the biological changes and the rate of the dark reaction appear to be of the same order of magnitude, so that the resultant deviations from reciprocity are a function of the two.

Energy-response curves are determined by a combination of (1) the functional dependence of pigment change on irradiation; (2) the distribution of "sensitivities" in the irradiated population to physiological response at a given pigment level; and (3) the range in amounts of the pigment required for a detectable response (a threshold concentration), on the one hand, and for saturation of response, on the other.

The probit (Finney, 1947) of the proportion of seed germinating varies linearly with the logarithm of the irradiance. This type of response is often encountered in biological assay but has not been explained. In fact, Eddy (1953) writes: "It seems rather that it must be treated simply as an approximate empirical relationship, expressing the fact that the range of variation of survival times is large in comparison with that often encountered in measurement of simpler properties." The precision of test is low, owing to relatively small populations, when the response is small or approaches saturation.

The number of pigment molecules in each cell or each object, such as a seed, is surely large (many powers of 10). A photoreaction progressively shifts this number, and at any one irradiance all seed are alike in the proportion of the pigment changed if the change is unimolecular with regard to energy and if the pigment is not variably screened by scattering or absorption. The objects might vary in their total pigment of both forms and in their response to a given concentration of effective pigment. All these variables appear as "sensitivities," and for seed germination "sensitivities" vary from germination response to very small irradiances to germination response requiring very large irradiances. Of greatest importance is the fact that an irradiance much greater than that required for essentially complete germination of seed does not markedly change the inhibitory irradiance required for 50 per cent response. In other words, saturation for germination approximately corresponds to complete conversion of the pigment from one form to the other. Some response, moreover, is noted for very little change.

In a unimolecular reaction, change of 90 per cent of the reactant takes about twenty times the energy required for a 10 per cent conversion. This is also approximately the case for seed-germination response upon irradiation. If, then, the 10 and 90 per cent conversions are used to establish a scale factor, the seed germination for intermediate irradiances follows an approximately unimolecular change.

The relative biological response, such as internode elongation, seed germination, or flowering, is limited by the photoisomerization of the

pigment. Sensitivity of the biological material, however, varies with conditions other than the state of the pigment. Thus for lettuce-seed germination the sensitivity varies with time of imbibition prior to irradiation (Borthwick, Hendricks, Parker, *et al.*, 1952). But the curves giving germination response as a function of irradiance are parallel. Energy-response curves for germination of tobacco and lettuce seed can be brought into approximate superposition by translation by a given factor in energy. This is the more remarkable when it is remembered that not only are the plants of different families, but also the temperatures, times

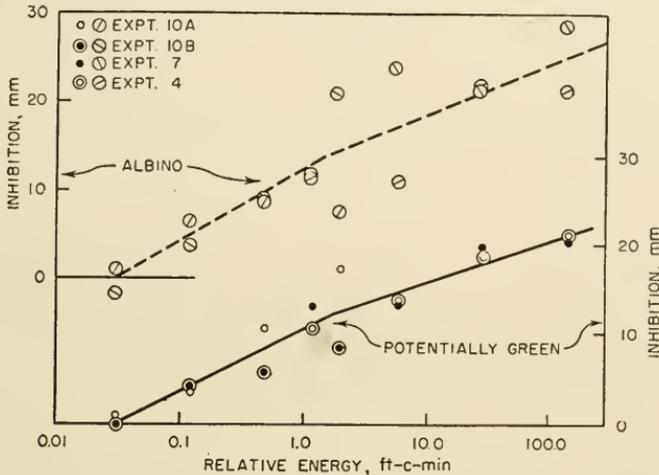


FIG. 10-3. Variation in inhibition in length of the second internode of dark-grown Colsees I barley seedlings with radiant energy. Population of albinos about 36; of potentially green, about 110. Results from four experiments are shown by the various symbols.

of imbibition, and durations of germination are different for the two kinds of seed.

The energy-response curves for floral initiation are quite different from those for seed germination (*ibid.*; Parker *et al.*, 1946). There appears to be a threshold energy for response, and an energy change of approximately fourfold is adequate to pass from a measurable response to saturation. Variance in response at a given energy is very small. These differences are compatible with a partial conversion of the pigment being necessary for initiation of response and with saturation being reached at some other intermediate value, such as 30 and 60 per cent conversion, respectively.

Variation of internode elongation of barley with energy (Borthwick *et al.*, 1951) is shown in Fig. 10-3. Essentially similar results were obtained for lengthening of leaves of dark-grown pea seedlings (Parker *et al.*, 1949). In stem lengthening the response to radiation is, to a considerable extent, inhibition of cell division (Avery *et al.*, 1937).

## CORRELATION OF SOME RESPONSES TO LIGHT

Whereas knowledge about the initial dark and light reactions is limited to some physical aspects, even less is known about the subsequent reactions in plants which are physiologically and morphologically expressed as flowering, bulb and tuber formation, stem elongation, and abscission. Since the effective materials are formed in the leaves and bring about changes elsewhere in plants, they well might be hormones. In particular, one material called "florigen" (Cajlachjan, 1937) has been postulated as controlling flowering. Hormones other than auxin which have been suggested by physiological responses are "vernalinalin" (Melchers, 1939; Melchers and Lang, 1941), "metaplasin" (Harder and Bode, 1943), and "flowering substance" (Struckmeyer, 1950). Vernalinalin was postulated to account for induced blooming of biennial *Hyoscyamus* plants in their first year. Its presence was later considered (Melchers and Lang, 1941) to be based on inadequate evidence. The "flowering substance" was said to be extracted from leaves of *Xanthium* and to induce flower formation when applied as a spray. Extraction of a flower-inducing material from a palm, *Washingtonia robusta*, inflorescence was also reported (Bonner and Bonner, 1948), but the material could not be obtained a second time. Progress on isolating active compounds is blocked by complete absence of assay methods. Since transfer of the stimuli has so far required contact of living tissue, the effective materials might not be very simple and could be protein.

Harder and Witsch (1940) noted that photoperiodic treatment of certain leaves that were not full-grown when the treatments were started resulted in modifications of their habit. Habits of leaves situated above the treated ones and in the same orthostichy but subjected to long photoperiods were also affected. These morphogenic effects were assumed by Harder and Witsch to result from action of a substance which they called "metaplasin" and which was produced in the leaf under the stimulus of short photoperiods. The pattern of effects produced in leaves above one subjected to short-day treatment led them to conclude that metaplasin was translocated through the vascular system. Harder and Gümmer (1947) obtained supporting evidence for this view by introducing berberine sulfate into the vascular tissue of the petiole of a certain leaf and noting that its distribution to structures above could be fairly accurately predicted on the basis of the morphogenic data. The work of Harder and his coworkers illustrates very well the fact that the photoperiodic reaction exerts control over a wide range of both vegetative and reproductive processes in the plant. Although they and others have been able to show that certain responses are experimentally separable and thus may result from different immediate control reactions, there seems to be no evidence that these could not stem from the same initial action of light.

Several theories have been advanced to correlate flowering responses as effected by photoperiodism. Some references to these theories are Bakhuyzen (1947), Bünning (1946), Cajlachjan (1937), Chouard (1943), Gregory (1948), Hamner (1942), Harder and Bode (1943), Lang and Melchers (1943), Lona (1946), Parker *et al.* (1948), and Withrow and Withrow (1944). In general, these theories separate the phenomena into several parts and postulate one or more active compounds. They are, however, little more than hypotheses to facilitate work. None is discussed here in detail, but some of the basic facts are presented.

Most of the theories postulate that a photoperiod preceding a dark period is required for formation of a specific active compound. That the photoperiod becomes less effective in promoting flowering when greatly shortened is shown by *Xanthium saccharatum* (Snyder, 1940) and soybean (Parker and Borthwick, 1940) for photoperiods shorter than 3 hr. It is also known in hundreds of other plants studied in less detail that a number of cycles of light and darkness will induce flowering. In fact, the term "photoperiodism" is closely identified with this behavior and was so conceived by Garner and Allard (1920). Several observations, however, indicate that the light period is required only for limiting the dark reaction and replenishing metabolic reserves when these are limiting. Thus controls of leaf and internode elongations in dark-grown seedlings drawing upon the metabolic reserves of the seed are effected with very low absorbed energy (Goodwin and Owens, 1948; Went, 1941). Flowering of *Kalanchoe blossfeldiana* (Harder and Gümmer, 1947), a plant with possibly high metabolic reserves, takes place when the photoperiod is only 1 sec in 24 hr with an intensity of 6000–8000 ft-c. In both these cases effect of light on a dark reaction alone is required. Albino plants of *Zea mays* have been brought to flowering and seed formation, in light of very low intensity, when supplied with sucrose (Spoehr, 1942). Infiltration of *Chenopodium amaranticolor* (Lona, 1950) roots and *Xanthium pensylvanicum* (personal communication from J. Bonner) leaves with sugar solution in darkness also resulted in floral induction.

Melchers and Lang (1942) found that *Hyoscyamus niger* would initiate flower primordia when held on 14-hr dark periods if the leaves were infiltrated with glucose, sucrose, fructose, or maltose. Control plants whose leaves were infiltrated with water remained vegetative. *Hyoscyamus* was observed to form flower primordia after adequate cold treatment even though the plants were completely defoliated and held in continuous darkness (Lang and Melchers, 1941). These facts led Melchers and Lang to postulate that certain processes in the leaves of long-day plants hinder floral initiation.

Other theories attempt to explain the apparently opposite flowering responses of long- and short-dark-period plants to the same initial photo-reaction. The identity of the initial reactions is best shown by the

various action spectra (Fig. 10-2). Similarly the action spectrum for inhibition of second internode lengthening of *Hordeum vulgare* (Borthwick *et al.*, 1951) is the same as that for enhancement of leaf development of *Pisum sativum* (Parker *et al.*, 1949). Other physiological responses are also to be considered in their bearing on the similarity between initial reactions in plants of different photoperiodic types. Moskov (1937) found that flowering in the long-dark-period Maryland Mammoth tobacco could be induced on short dark periods by grafting to it leaves from *Nicotiana rustica*, which flowers independently of day length. Similar results were obtained with *Soja max* var. Biloxi, upon which leaves of the variety Agate were grafted (Heinze *et al.*, 1942). This latter variety is also sometimes considered to be indeterminate, but it too is dependent on long dark periods in that such periods give a more pronounced flowering response. Control of flowering in plants of one photoperiodic type by leaves of the opposite type was obtained by Melchers and Lang (1941) in the case of Maryland Mammoth tobacco and *Hyoscyamus niger*. Experiments of this kind strictly require only that the floral-inductive materials of short- and long-dark-period plants be effective for both types, but generalization that a single material is involved is warranted as a working hypothesis. Interspecific effects are also found in the dependence of flowering of the parasitic species *Orobanche minor* on that of one of its hosts, red clover (Holdsworth and Nutman, 1947).

Reaction of certain strawberry varieties to the length of the dark period affords an example of two distinct responses. Formation of flower buds occurs only when the plants are subjected to long dark periods. If the dark periods are shortened or are interrupted near the middle, flower-bud initiation is inhibited, but runner formation is promoted. Sexual reproduction of strawberry is thus induced by long nights, and asexual reproduction, by short nights. Flower formation in strawberry occurs first at the terminal of the main stem, its meristem forming a terminal inflorescence. Following this the terminal meristems of buds in the axils of leaves also produce inflorescence primordia. If photoperiodic conditions are not conducive to flower-bud formation, these axillary buds may develop as branch rosettes having very short stems and producing a succession of vegetative leaves, or they may produce runner plants at some distance from the parent plant through the great elongation of the first two internodes. Flower and runner formation thus represent the extreme range of response of axillary buds to different photoperiodic treatments.

Variation within a species from short- to long-dark-period response might also be indicative of control by the same initial reaction. Such variation has been observed in a grass, side-oats grama, *Bouteloua curtipendula* (Olmsted, 1945). Cases of less extreme variation from varieties

of definite responses to those with indeterminate ones are shown by tobacco and strawberry.

A way in which these various responses can be correlated is schematically shown in Fig. 10-4. It is based on the idea of limited effective concentration ranges for some material that at low values is inadequate for action and is inhibitory above an upper concentration limit.

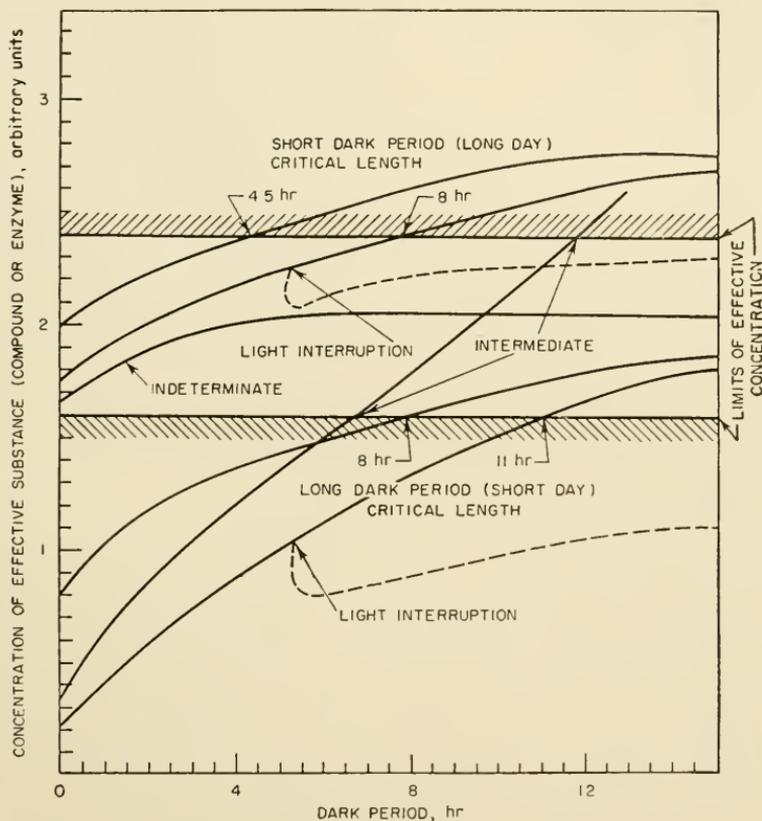


FIG. 10-4. A scheme illustrative of photoperiodic response.

### PHOTOPERIODIC AFTEREFFECTS

Aftereffects of an inductive period consisting of one or more cycles of light and darkness, followed by return to noninductive flowering conditions, have been studied. Several questions are involved. How does subsequent development depend on the degree of induction? Is the leaf modified so as to continue its inductive action under adverse dark periods? Is there storage of an effective material that gradually undergoes dilution?

Information on the first question has been classified as "photoperiodic induction" (Maximov, 1929) and "photoperiodic aftereffect" (Moskov,

1941). Several conditions, described from experiences of the authors, might be met in subsequent development when a florally induced plant is returned to an adverse photoperiod: (1) floral buds might fail to develop, as in spinach, or abort, as in soybean; (2) incompletely formed flowers might result, as in some varieties of sugar beet and *Chrysanthemum*; or (3) the plant might continue to flower, as in *Xanthium saccharatum*, or resume vegetative growth, as in soybean.

There is a rapid recovery from the flower-promoting effects of long dark periods on Biloxi soybeans; thus two successive photoinductive cycles induce formation of flower primordia, but ten or more may be required if each is alternated with a cycle unfavorable for flowering. The effects of partial induction are well shown by development of *Euphorbia pulcherrima* when grown under dark periods near the critical length (Parker, Borthwick, and Rappleye, 1950). Flowers develop with bracts that are mottled green and red instead of intensely red, as on plants that are adequately induced. *Perilla* (Moskov, 1939a) is another example of a plant whose flower development is markedly dependent on the length of the dark period.

Short-day species and varieties differ greatly in the extent of their recovery from effects of long dark periods; a species of cocklebur, *Xanthium pensylvanicum*, is an extreme case. A single dark period of adequate length is sufficient not only to induce flower-bud formation in this plant but also to enable the flowers to complete their development (Hamner and Bonner, 1938). Lona (1947) found, however, that some species of cocklebur are less responsive. A tetraploid race of *X. italicum*, for example, required a minimum of two successive photocycles for floral induction if the night lengths are about 15 hr and of more than two if they are about 9 hr. The diploid form of the same plant regularly responded to a single long dark period. Lona (1946) also observed that *X. orientale*, although a long-dark-period plant in most respects, could not be prevented from flowering by application of continuous light. Similar observations were made for soybean varieties such as Agate and Batorawka (Borthwick and Parker, 1939). Such a range of behavior is probably of frequent occurrence in other groups of related varieties of plants.

The second question of possible leaf modification has been studied by grafting "induced" leaves or branches on vegetative stocks or scions and subsequently maintaining the entire plant under dark periods adverse for flowering. Moskov (1939b) and Cajlachjan and Yarkovaja (1937), working with *Perilla*, found that a vegetative scion was induced to flower when grafted to an induced stock. Similar results were obtained with *X. pensylvanicum* by Hamner and Bonner (1938) when approach grafts were made between induced and noninduced plants. This was not the case for *Soja max* var. Biloxi (Heinze *et al.*, 1942). The persistence of

flowering response discussed in this and the preceding paragraph appears to be an expression of the capacity of the plant to continue formation or development of floral primordia upon return to noninductive photoperiods.

Many plants that are photoperiodically responsive when young later appear to become more indeterminate. Examples are the long-dark-period *Glycine max* var. *Biloxi* and *Kalanchoe blossfeldiana*, which upon continued growth eventually differentiate flower primordia in short dark periods. The short-dark-period plant *Baeria chrysostoma* (Sivori and Went, 1944) maintains its photoperiod definitiveness for only a short time. An extreme case of continued vegetative growth is afforded by *Sedum spectabile* maintained on long dark periods for 9 years and by *S. woodwardii* maintained for 8 years, both of which were brought to flowering in a few weeks on short dark periods (Garner and Allard, 1931b).

Photoperiodic control is closely associated in some animals with the pituitary function, which apparently is secondary to the primary stimulus. Hypophysectomy of the ferret (Bissonnette, 1938) and the duck (Benoit and Ott, 1944) prevented gonad development under favorable photoperiodic cycles, thus demonstrating the intermediary action of the pituitary hormones. Removal of the gonads of the varying hare (Lyman, 1943), on the other hand, did not stop the change in coat color, nor did similar treatment of crows interfere with their southward migration in autumn (Rowan, 1946). Knowledge of hormonal control in arthropods and other invertebrates is less developed, and the organs controlling sexual function are still unknown in many cases.

Under photoperiodic conditions that are not entirely adequate for full sexual development, animals are partially stimulated. This was shown to be the case for ferrets (Bissonnette, 1932a), starlings (Burger, 1949), and sheep (Yeates, 1949). Whether aftereffects in the sense discussed for plants occur in animals is still unknown. In many birds such as the turkey, *Meleagris gallopavo*, the production of one or more clutches might follow a photoperiodic stimulus (Scott and Payne, 1937). The aftereffects could be the continued functioning of the induced pituitary, but the information available is too limited to attempt correlations. In animals the chain of events between perception and stimulation of the pituitary or other control organ is unknown, as it is for plants. In plants the organ of perception is often still enlarging, and for this reason it might be more susceptible to permanent modification of functions that control reproduction by suitable photoperiodic conditions than are the organs of perception of animals that respond cyclically through a multiplicity of reactions in the mature organism.

Some animals are like plants in that, although photoperiodically sensitive, they may still become sexually active if held for long periods under adverse photoperiodic conditions, as was found for ferrets by Bissonnette (1932a). The potato aphid, *Macrosiphum solanifolii* (Shull, 1929), by

contrast continues for many generations to produce flightless forms parthenogenetically and still can be quickly induced to produce winged migrant forms (Bissonnette, 1932a). This apparently is equally the case for other aphids that reproduce parthenogenetically for long periods and then can be induced to produce sexual forms (Marcovitch, 1924).

#### FLOWERING IN RELATION TO AUXINS

Floral initiation in long-night plants can be suppressed by continued applications of auxins and enhanced by use of antiauxins (Bonner and Bandurski, 1952). Bonner and Thurlow (1949) found that indoleacetic acid and  $\alpha$ -naphthaleneacetic acid at a concentration of 500 mg/liter suppressed floral initiation of *Xanthium canadense* (*X. chinense*) when sprayed on the foliage. Cuttings placed in nutrient solutions of  $\alpha$ -naphthaleneacetic acid at concentrations of 10–100 mg/liter and held in short photoperiods were inhibited in floral initiation. It was necessary to apply the auxins only during the dark period, and they were most effective at the beginning of darkness. *Xanthium canadense* leaves given an external supply of auxin showed little increase in auxin by the *Avena* test. Cajlachjan and Zdanova (1938) concluded that auxins as measured by the *Avena* test were formed more intensively on long photoperiods for all types of plants. Existence of antiauxins and inhibitions, however, confound the *Avena* test.

The effects of  $\alpha$ -naphthaleneacetic acid on the short-night plant Wintex barley and the long-night plant Chalco teosinte were studied by Leopold and Thimann (1949). Growth regulators did not induce floral initiation when applied to either of these plants maintained on unfavorable photoperiods. Wintex barley maintained on photoperiods favorable for floral initiation responded to very low concentrations of  $\alpha$ -naphthaleneacetic acid by an increase in the number of flower primordia as contrasted with control plants. Higher concentrations of  $\alpha$ -naphthaleneacetic acid, however, inhibited the formation of floral primordia. Applications of growth regulators to teosinte maintained on photoperiods favorable to floral initiation resulted in inhibition of flowering at all concentrations tested. Increased growth of Wintex barley was strongly correlated with increased number of flower primordia. Stem elongation of barley has also been shown to be closely correlated with spike development (Borthwick, Hendricks, and Parker, 1948).

Denffer and Grundler (1950) observed that growth in height of *Sinapis alba*, *Fagopyrum sagittatum*, *Impatiens balsamina*, and *Calendula officinalis* was reduced and blooming time appreciably delayed following treatment of leaves with very low concentrations, 0.005–0.02 per cent, of  $\alpha$ -naphthaleneacetic acid and  $\alpha$ -indoleacetic acid. In all these species except *C. officinalis*, however, flowers formed at approximately the same node

on the treated plants as on the controls. With *C. officinalis* more nodes were formed below the inflorescence on the main stem of the treated plants than on that of the controls.

Floral initiation of the short-night plants *Silene armeria* and annual *Hyoseyamus niger* were found by Liverman (1952) to be promoted by auxins applied to plants that were held on photoperiods near the threshold for response. *Silene* plants sprayed with indoleacetic acid solutions elongated much sooner than untreated controls, and there appeared to be a relation between the amount of supplementary light and responsiveness to applied auxin.

Control of the length of the first internode of barley is effected by light of the same quality as that for control of floral induction. Mer (1951) found that growth of the mesocotyl of *Avena sativa* did not depend on the auxin diffusing from the coleoptile tip. The length of this structure is strongly controlled by light, and perception is not affected by the action of cyanide or iodoacetate or by anaerobic conditions. The effects of illumination survive a period of drying and become apparent upon subsequent germination of the grain in darkness. Apparently this effect is on the embryo, since perception occurs in embryos excised from dry grain and grown on culture medium. Mer concluded that auxin itself was not the primary reactant in the perception process.

General observations on the effects of growth-regulating compounds has been determined for different plants by several investigators (Bonner, 1940; Clark and Kerns, 1942; Cooper, 1942; Leopold and Thimann, 1949; Van Overbeek, 1946). Clark and Kerns (1942), working with the pineapple, *Ananas comosus*, reported that  $\alpha$ -naphthaleneacetic acid applied as a foliage spray in concentrations of 0.1 mg/liter hastened floral initiation, whereas tenfold higher concentration delayed the process. They pointed out that, even though this compound affects floral initiation, it is not to be considered as a "florigen" but is to be classed with acetylene and ethylene, which also induce premature flowering in *Ananas*.

Cooper (1942), working with the Abachi variety of pineapple in Florida, reported that treatment with  $\alpha$ -naphthaleneacetic acid in October induced premature flowering, whereas treatments made in July had no effect. Ethylene, however, induced premature flowering equally well in summer and fall. In these experiments separate lots of plants were sprayed with  $\alpha$ -naphthaleneacetic acid,  $\alpha$ -naphthalene acetamide, and  $\beta$ -indoleacetic acid in concentrations of 0.01, 0.005, and 0.001 per cent. Cooper's results with 0.01 per cent  $\alpha$ -naphthaleneacetic acid differed considerably from those of Clark and Kerns (1942) in Hawaii in that the treatment hastening flowering in Florida delayed flowering in Hawaii.

Van Overbeek (1945, 1946) worked extensively with pineapple in Puerto Rico. He observed that solutions of  $\alpha$ -naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid applied to the crowns of vegetative plants

at a concentration of 5 ppm, or 0.25 mg per plant, were adequate to cause floral initiation in any month of the year. Distribution of free and bound auxin in vegetative Cabezona pineapple plants was measured by Van Overbeek *et al.* (1947); the free auxin content was found to be greatest in the apex of the stem axis, and the base of the youngest leaves had the highest amount of bound auxin. On the basis of these data the hypothesis was advanced that flowering under natural conditions was brought about by an increased level of free auxin in the apical meristem, resulting from an increased rate of conversion of bound auxin from the young leaf bases into its free form. Low night temperatures were observed by Van Overbeek and Cruzado (1948b) to cause unseasonable flowering of Red Spanish pineapple, a variety reported as insensitive to photoperiodic control. Geotropic stimulation (Van Overbeek and Cruzado, 1948a) also resulted in floral initiation on the Cabezona variety but was ineffective on the Red Spanish and Smooth Cayenne varieties. These observations indicate that floral initiation in *Ananas comosus* might be caused by a common effect of various treatments on the physiology of the plant. Van Overbeek pointed out that auxin level can be an important internal factor, which in itself need not be causative but may interact closely with other processes that influence floral initiation. This may be similar to the functioning of the pituitary in photoperiodically sensitive animals.

The auxin economy of the plant thus has been demonstrated to interact with floral initiation and flower development. How causative it might be as a control factor, however, remains to be established.

#### INHERITANCE OF RESPONSIVENESS TO PHOTOPERIOD

The genetics of photoperiodism might serve as a guide for physiological and possible biochemical work. Action of a single gene is thought to control a particular reaction even though the reaction involves functioning of many genes. If a single gene is involved in inheritance of photoperiodic response, it might possibly affect the photochemical reaction.

The most extensive investigations of inheritance of photoperiodic response have been with tobacco. Allard in 1919, before the discovery of photoperiodism, described crosses of Maryland Mammoth tobacco with White Burley, Connecticut Broadleaf, and several other varieties of *Nicotiana tabacum* which are now known to be indeterminate. In all cases the nonflowering characteristic of Maryland Mammoth as grown under field conditions was inherited as a unit character. A total of 1820 F<sub>2</sub> plants, of which 439 were of the Maryland Mammoth type, were grown. Lang in 1948 made crosses between Maryland Mammoth and the indeterminate variety Java. Of a total of 467 F<sub>2</sub> plants, 128

were found to be of the long-night type. The inheritance ratios closely approach 3:1 in both cases.

Inheritance of photoperiodic response in *Sorghum vulgare* varieties is also controlled by a single gene, *Ma*, which is modified in its expression by two others, *Ma*<sub>2</sub> and *Ma*<sub>3</sub> (Quinby and Karper, 1945). The genes *Ma*<sub>2</sub> and *Ma*<sub>3</sub> are dependent for their expression on the presence of dominant *Ma*. Quinby and Karper found, in the F<sub>2</sub> progeny of a cross between the intermediate maturing SA5484 Dwarf Yellow milo variety and the early-maturing Sooner milo, that 46 of 192 progeny were early-maturing. Genetically the cross was *Ma ma*<sub>2</sub> *ma*<sub>3</sub> × *ma ma*<sub>2</sub> *ma*<sub>3</sub>. Early maturing corresponds to flowering under short night lengths and is an indeterminate expression for sorghum. Single-gene control of inheritance was also observed for crosses between the long-night teosinte, *Euchlaena mexicana*, and *Zea mays*, which is only weakly responsive to long nights (Langham, 1940).

Inheritance of photoperiodic response in short-night plants has also been found to be controlled by a single gene where tested. Bremer (1931) and Bremer and Grana (1935) found this to be the case for *Lactuca sativa*; the crosses were between the short-night winter lettuces and the indeterminate summer varieties. In the F<sub>2</sub> generation of a cross between a winter variety, Maiking, and a summer salad variety, Deacon, 1092 of 1473 plants resembled the Maiking parent in flowering early in the summer on short nights. Similar results were reported for another short-night plant, *Epilobium hirsutum* (Ross, 1942).

In tobacco the indeterminate photoperiodic response is partially dominant over the long-night response (Lang, 1948). This is also possibly the case for teosinte (Langham, 1940) and for some varieties of *Tagetes erecta* (Little *et al.*, 1940) in which the long-night character is also recessive. In sorghum varieties, however, the long-night character is dominant. The short-night character of *Lactuca sativa* and *Epilobium hirsutum* is dominant over the indeterminate.

The ecological variation due to night length observed for animals would suggest that the photoperiodic factor is expressed. A work possibly bearing on the subject is that of Hafez (1950) on variation in the sexual seasons of three breeds of sheep. The breeding season of ewes originating in Scotland was in the period when the nights were 11.5–12.5 hr or more in duration. Night lengths during the breeding season for a breed originating in southern England varied from 10.5 to 12 hr and were shorter still for breeds having merino blood.

The photoperiodic responses of the several plants studied in detail thus indicate that a single gene effects control. The factor and its alleles could well differ more in a quantitative sense in their influence on response than in qualitative distinction. This would be in harmony with the scheme shown in Fig. 10-4, in which all plants are considered as basically

similar in their photoperiodic mechanism but quantitatively distinct. It would follow that, in biochemical work undertaken for the purpose of isolating dark-period products peculiar to photoperiodism, the best source might be a short-night plant subjected to long nights.

#### INFLUENCE OF ENVIRONMENTAL FACTORS ON PHOTOPERIODIC RESPONSE

Photoperiodic stimulus is a very important factor in the ecology of plants and animals. This aspect of the subject has been discussed by Allard (1928, 1932), Baker (1938), Baker and Ranson (1938), Bünning (1948), Garner (1936), Henfrey (1852), Marshall (1936), Rowan (1938), and Sircar (1938). It will not be considered here in the many aspects of speciation and type of response, but rather attention will be restricted to the causative factors.

The variation in night length with latitude and season is of interest (Fig. 10-5). At 10° from the equator the maximum variation in night length is about 65 min. Even within this small variation, effects that seem to be photoperiodic have been reported in plumage change of birds (Moreau *et al.*, 1947), breeding habits of lions and other mammals (personal communication from C. E. Kellog, of the U.S. Department of Agriculture, based on information obtained in the Belgian Congo), production of rice (Kerling, 1950; Sircar, 1938), and flowering of sugar cane (Sartoris, 1939). The rate of change of night length is also smaller near the equator than in higher latitudes. This rate of change might be an important factor for the intermediate plant sugar cane, in which flowering appears to depend upon whether the critical period is approached from long days of summer or short days of winter (personal communication from G. O. Burr, of the Hawaiian Sugar Planters Association, based on observations on sugar-cane production in Hawaii).

Diurnal and seasonal temperatures and changes in temperature might interact both with the final physiological expression and with translocation from the region of perception. Effects of temperature, within the range of biological activity, on the primary photoreaction are unlikely unless the concentration of the initially absorbing compound is temperature-dependent. Lack of dependence of the initial photoreaction on temperature during a short interruption of a dark period with light was established for *Kalanchoe blossfeldiana* (Harder *et al.*, 1944). Similarly Parker and Borthwick in unpublished work found that temperatures between 15° and 40°C applied during dark-period interruptions had no significant effect upon response of Biloxi soybeans.

Effects of temperature variation on the over-all photoperiodic response have been measured for floral induction of soybean and bulbing of onion. These temperature effects are largely on the dark-period reaction. Floral

initiation of Biloxi soybean, growing on favorable photoperiodic cycles, is less at 13° and 30°C than at intermediate temperatures for the dark period irrespective of light-period temperatures between 13° and 30°C (Parker and Borthwick, 1939). This temperature effect is on the leaf blade, which is the organ of perception (Parker and Borthwick, 1943). That the effect was not on translocation was demonstrated by cooling

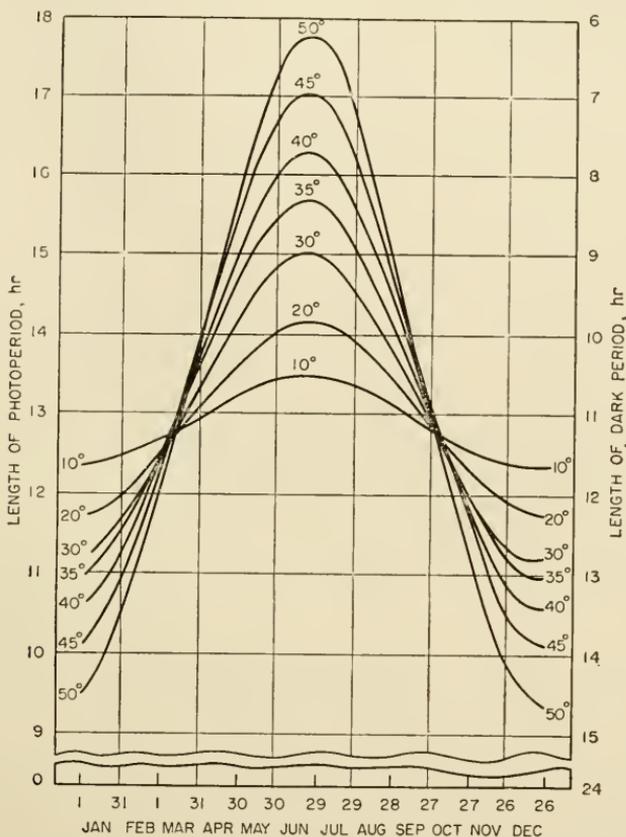


Fig. 10-5. Light and dark periods at 10°–50° north latitudes. Light periods include morning and evening twilight, beginning and ending, respectively, with the sun 6° below horizon. (Data from *American Nautical Almanac*, 1943, U.S. Naval Observatory.)

the petioles of leaves receiving photoperiodic cycles favorable to flowering (Borthwick *et al.*, 1941). It was necessary to cool the petiole to 4°C to block the response, and at this temperature the transport of other metabolic products was blocked. Flowering of *Allium cepa* is inhibited by high temperatures under all photoperiodic conditions (Heath, 1943; Holdsworth and Heath, 1950). Bulb formation in *A. cepa*, which involves cessation of mitoses in the primordial leaf tips and consequent production of bladeless scale leaves (Heath and Holdsworth, 1948), is also markedly temperature-dependent, being favored by short dark

periods and high temperatures. Dark periods just adequate for bulbing at high temperatures (Heath, 1943) do not permit bulbing at lower temperatures.

In the case of warm-blooded animals, environmental temperature seems to play a minor part in sexual reproduction. The striking response of egg laying at temperatures far below 0°C on artificially lengthened days in winter in some of the first experiments with juncos (Rowan, 1926) and later work with pheasants (Bissonnette and Csech, 1941) emphasized dark period rather than temperature as a controlling factor. In other animals such as aphids, interaction between temperature and dark period is possibly more pronounced but still not controlling. For instance, in the green apple aphid (Marcovitch, 1924) this might be a factor determining the number of parthenogenetic generations preceding migration in the spring.

Some attention has been turned to the effect of rate of change of day length on sexual response. In fact, the general practice in experiments with animals has been to simulate the seasonal change in day length (Bissonnette, 1932a; Rowan, 1926). Photoperiodically sensitive plants and animals readily responded to abrupt interruption of dark periods, in so far as they have been tested, as well as to either abrupt or gradual change in the length of the photoperiod used in experiments. The actual way, however, in which the length of the dark period changes with season and with latitude might have an important interaction on photoperiodic response of both plants and animals. The behavior of soybean affords an example. The long-night Biloxi variety when shifted abruptly from 8- to 16-hr dark periods may form as few as three or four flowers in the terminal inflorescence. Plants that pass through the critical length of dark period under natural conditions, however, might have several times this number. This same behavior has been observed in other soybean varieties grown in two latitudes, the more southern one having the greater number of flowers in its racemes. The more gradual change in night length in the southern latitudes results in a longer time spent in the critical photoperiodic region. Under these conditions the terminal meristem is able to function over a longer period before the night lengths increase sufficiently to prevent further differentiation. There is similarly a time course in the sexual development of animals which depends upon the intensity of photoperiodic stimulation. This has been studied somewhat by Bissonnette, working with *Sturnus vulgaris* (Bissonnette, 1931), and by Marshall (1950). The rate of change of day length has also been mentioned as a possible factor interacting with photoperiod in its effect on bird migration (Allard, 1928); however, Bissonnette (1936b) commented that migration might result from cycles of the anterior lobe of the pituitary.

Light intensity during the photoperiod when adequate to give moder-

ate photosynthesis is not a factor in floral induction (Snyder, 1940). Photoperiodic control of flowering is effected by radiant energies far lower than these, whether they are used to extend a natural photoperiod or to interrupt a dark period.

### REVIEWS ON PHOTOPERIODISM

Interest in the subject of photoperiodism is world-wide, and articles are disseminated in the journals of many countries. Since the phenomenon is exhibited by both plants and animals, articles are likely to appear in almost any journal devoted to natural science. For this reason reviews by authors in various countries and fields of activity are of special value. Articles on photoperiodism in plants have been reviewed at frequent intervals, beginning as early as 1925. Garner discussed the subject in 1936 and listed several of these earlier reviews. The literature was well summarized again in the period 1936-1939 by Burkholder (1936), Cholodny (1939), and Murneek (1937). More recent reviews include those by Borthwick, Parker, and Hendricks (1948, 1950), Chouard (1949), Hamner (1942, 1944), Lang (1949), Melchers and Lang (1948), Murneek (1948), and Parker and Borthwick (1950). Samygin (1946) gave extensive lists of plants grouped by families, showing the nature of the photoperiodic response and a reference to the original work. Review papers dealing with photoperiodic responses of animals include those by Bissonnette (1936a), Burger (1949), Jenner (1951), Marshall (1936), Rowan (1938), and Yeates (1949).

### COGNIZANCE

The significance of the many observations mentioned in the several sections is only suggested, for each work is circumscribed. Does a pattern exist and is knowledge adequate to permit even the barest outline to be measured? The outline is taking shape, and photoperiodism, instead of being only a minor phenomenon, is one expression of a primary process controlling development. The nature of the primary process is essentially established, but the following steps by which control is effected are still unknown, though subject to experiment.

How universal is the phenomenon? In bold summary, a similitude exists in control by darkness of reproduction in plants and animals, even though the course for each is unique. The control affects other functions that can best be classed together as development. The initial reaction takes place in cells of one part of the plant or animal and affects development of remote parts. Control is shown both in highly developed forms, such as the chordates, and in more primitive ones.

The control mechanism involves a dark reaction that is recognized in

extreme forms by the counteracting effect of light. If the counteraction of light is incomplete or absent, the dark reaction might pass unrecognized in the plant or the animal. This might also be the result if the control system, once adequately established, modifies the organ of perception so as to ensure the continuance of the induced chain of reactions. Indeterminate, or day-neutral, forms, which might be considered as developing equally well in continuous light or ordinary natural cycles of light and darkness, could in these or related ways still be controlled by the same initial reaction as responsive forms, but pass unnoticed. Indeed the great variation in presence and degree of physiological modification in response to darkness is evidence of the universality of the response.

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## CHAPTER 11

# Seed Germination

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*Introduction: Historical—Definitions of terms used. Photoblastism as a function of external conditions: Temperature—Quantity and quality of light—Chemicals. Photoblastism as conditioned by pretreatment: Photo- and skotodormancy—Pretreatment with different temperatures—Presoaking and drying—Climatic conditions during ripening of seeds. Photoblastism as a function of inner conditions: Influence of coats enveloping embryos—Afterripening—Photoblastism and metabolism. Conclusions: Summary of facts—Theories—Suggestions for further work. Addendum. References.*

### 1. INTRODUCTION

#### 1-1. HISTORICAL

Caspary (1860) was the first botanist to observe the beneficial influence of light on germination. But it was not until 1881 that Stebler (1881a,b,c) investigated the problem of light germination in a systematic manner. Using the achenes of different grasses, he proved that the grains of a number of grass species germinate much better in light than in darkness. In 1903 Heinricher reported the first case of germination inhibition caused by light (*Acanthostachys strobilacea*); his paper was followed in 1904 by a paper by Remer, who showed that the seeds of *Phacelia tanacetifolia*, which have since been used frequently, germinate better in darkness than in light.

Since then the research on the influence of radiation on germination has developed along two different lines. On the one hand, agriculturists, seed testers, and others have reported a steadily mounting number of species whose germination is influenced by light (reviews given by Kinzel, 1907 to 1915b; Niethammer, 1928, 1934). Besides being of considerable practical importance, these investigations have shown that, far from being isolated curio cases, the effect of light upon germination is a widely spread biological phenomenon. This is clearly shown by the very large number of species whose germination is influenced by light, as well as by the fact that, when investigated carefully, such seeds as wheat, corn, and rye, whose germination had been considered to be indifferent to light, were shown to be photosensitive (Burgerstein, 1913; Niethammer, 1928;

Mosheov, 1938; Chrismar and Fernando, 1942). On the other hand, various investigators have tried to reveal the mechanism of the light effect. Joensson (1893), Lehmann and associates (various papers, 1909 to 1931), Gassner (various papers, 1910 to 1930; Gassner and Franke, 1934-1935), and their numerous coworkers have tried to find out how light acts upon germination. This causal research received a new stimulus when, through the development of a better experimental technique, detailed action spectra of light germination became possible (Kommerell, 1927; Flint, 1934, 1935, 1936; Flint and McAlister, 1935, 1937; Meischke, 1936; Resühr, 1939a,b).

But, though a mass of interesting experimental data has been collected, we are still far from a real understanding of the influence of light on germination [for detailed historical surveys see Lehmann (1915), Gardner (1921), Lehmann and Aichele (1931), Crocker (1936)].

## 1-2. DEFINITIONS OF TERMS USED

A clear-cut terminology is, just as a material tool, an instrument of research. Since different authors use different terms in describing the same fact and since sometimes the same term is used in a completely different sense by different authors, we give first of all a definition of all the terms used in this chapter.

*Dormancy:* Any condition of perfectly viable seeds which makes them resistant to germination under environmental conditions that are ordinarily favorable for quick germination (Toole, 1939). When dormancy is caused by the presence or absence of light, we speak about photo- or skotodormancy (from the Greek *skotos* = darkness); when caused by temperature conditions, about thermodormancy; and when caused by chemical agents, about chemodormancy.

*Afterripening:* Naturally occurring changes which take place in seeds after harvest and which bring about changes in their dormancy (*ibid.*).

*Photoblastism:* The influence of the presence or absence of light on the germination of certain seeds. Photoblastism may be positive or negative, depending on whether the light stimulates or inhibits germination.

*Photorequirement:* The need for light of positively photoblastic seeds.

*Photosensitivity:* The relation between the quantity of light and the percentage of germination of photoblastic seeds.

*Inhibiting light:* Light of a certain wave length which depresses the germination percentage below that of darkness. When given after stimulating light, it depresses the percentage of germination below that obtained with stimulating light alone.

*Stimulating light:* Light of a certain wave length which increases the percentage of germination above that of darkness. When given after inhibiting light, it increases the percentage of germination above that obtained with inhibiting light alone.

*Indifferent light:* Light of a certain wave length which acts like darkness. When it is given after stimulating or inhibiting light, the percentage of germination remains unchanged.

*Sensibilization and desensibilization:* All processes that lead to a higher or lower photosensitivity. We speak about photo-, thermo-, and chemosensibilization, according to the causative agent.

## 2. PHOTOBLASTISM AS A FUNCTION OF EXTERNAL CONDITIONS

### 2-1. TEMPERATURE

Photoblastism is greatly influenced by temperature, as has been pointed out by Pauchon (1880) and Cieslar (1883). This was confirmed by many others (Gassner, 1911a,b, 1915a; Baar, 1912; Lehmann and Aichele, 1931; Kincaid, 1935; Thompson, 1938; Resübr, 1939a; Evenari, 1952).

*Chloris ciliata*, a South American pampas grass extensively studied by Gassner (1911a,b) is positively photoblastic at higher temperatures, non-photoblastic at 20°C, and negatively photoblastic at temperatures below 20°C.

Three species of *Amaranthus* and *Physalis franchetti* behave in the same way (Baar, 1912). For *A. caudatus*, for example, the percentages of germination at different temperatures in light and darkness are as follows:

Temperature, °C	Percentage of Germination	
	Light	Darkness
5	0	10
10	10	35
20	35	48
25	33	33
35	38	29
40	37	0

This example shows clearly that the terms "positive photoblastism," "negative photoblastism," and "photo-indifferent" cannot be used for classifying seeds, since the photoblastism of one and the same species and of one and the same sample is dependent upon external and internal conditions. These terms designate only a certain physiological state. As additional proof may be cited the fact that the same sample of seeds is stimulated or inhibited by light at different stages of afterripening (see Sect. 4-2).

Whereas for most photoblastic seeds temperature is an important factor in determining their germination behavior toward light, the relation may be completely different from that found for *Chloris*, *Amaranthus*, and *Physalis*. Thompson (1935, 1938) reported that lettuce seeds are positively photoblastic only through a temperature range of 18°-25°C. At temperatures below 18°C they become indifferent to light. This is too general a statement, since different varieties behave differently. Lettuce

seeds var. Grand Rapids germinate at 14°C with the same high percentage in both light and darkness (Evenari, 1952). As the temperature rises above 14°C, the seeds get more and more positively photoblastic; i.e., with rising temperatures, germination is more and more inhibited in darkness. This inhibition is overcome by light. Lettuce seeds var. New York are nonphotoblastic between 14° and 24°C. At higher temperatures they become negatively photoblastic (*ibid.*).

*Epilobium hirsutum* and *E. roseum* react still differently. The lower the temperature, the more positively photoblastic they are. At higher temperatures they become indifferent to light (Gassner, 1915a). This is contested for *E. hirsutum* by Fassbender (1925), who found that with rising temperatures these seeds do not lose their photoblastism but only become more photosensitive.

*Phacelia tanacetifolia* is negatively photoblastic at all temperatures, whereas five *Oenothera* species (Gassner, 1915a), *Primula obconica*, and *Raymondea pyrenaica* (Schroeder and Barton, 1938-1939) were positively photoblastic over the whole temperature range over which germination occurred.

Alternating temperatures mostly have a very pronounced influence upon the germination of photoblastic seeds (e.g., Liebenberg, 1884; Gassner, 1915a; Fassbender, 1925; Kincaid, 1935; Andersen, 1947). Liebenberg (1884) even went so far as to identify the effect of light and alternating temperature when he found that *Poa pratensis* did not germinate at 20° and 30°C without light but germinated well in darkness with temperatures alternating daily between 20° and 30°C. The germination of *Ranunculus sceleratus* is brought about by light only when the illumination is accompanied by intermittent temperatures. Light without intermittent temperatures is more or less ineffective, and intermittent temperatures without light are less effective than with added light (Gassner, 1915c). When alternating temperatures of 12°C for 4 hr and 28°C for 20 hr daily were used, the percentage of germination of *Oenothera biennis* reached 90 per cent in the dark, whereas the dark germination at 12° and 28°C was 0 and 65 per cent, respectively. This means that intermittent temperatures had exactly the same influence as light in overcoming the germination inhibition observed at constant temperatures in darkness, since at constant temperatures of 28°C in light the percentage of germination reached only 91.5 per cent (*ibid.*). With *Lactuca* Thompson (1938) observed that an alternation of temperature between 5°-10°C and 22°-30°C brought about germination in the dark more effectively than did low temperatures alone. But with *Phacelia* intermittent temperatures are ineffective (Gassner, 1915a).

An interesting feature of some seeds is the thermodormancy caused by high temperatures. At temperatures of 30°-35°C lettuce seeds enter a state of deep dormancy which cannot be overcome by light (Borthwick

and Robbins, 1928; Thornton, 1936; Thompson, 1938). This thermodormancy can be broken only by low-temperature treatment (Davis, 1924; Borthwick and Robbins, 1928; Thornton, 1936) or by removing or pricking the endosperm together with the integumentary membrane, which is inseparable from it (Borthwick and Robbins, 1928; Leggatt, 1948).

Summing up, we may say that temperature very largely determines the photoblastic reaction of seeds, but different plants react differently. The only common feature may perhaps be found in the fact that, at the thermal optimum of germination, photoblastism is at its minimum. The farther away we get from this optimum, the more pronounced becomes the photoblastic reaction (Resüher, 1939a,b). If this is so, many seeds considered as indifferent to light will in the future be found to be photoblastic when experimented with at nonoptimal temperatures (*ibid.*).

## 2-2. QUANTITY AND QUALITY OF LIGHT

*Photosensitivity.* Photoblastic seeds differ very much in their photosensitivity. *Lythrum salicaria* still reacts to 730 HK (Hefner Kerzen; 730 HK = 657 ft-c) applied for 0.1 sec at 30°C (Lehmann, 1918). Tobacco seeds show a significant increase in germination when illuminated for 0.01 sec with direct sunlight. They are so photosensitive that strong moonlight applied for 15 min stimulates germination (Kincaid, 1935). The germination of *Phleum pratense* is stimulated by 200 MK (Meter-Kerzen; 200 MK = 18.5 ft-c) of white light applied for 1 sec (Maier, 1933b).

For *Lactuca* var. Grand Rapids 0.2 sec of 250 ft-c at 26°C is the minimum quantity of light which produces a statistically significant stimulation of germination (Evenari, unpublished observations). In contrast to these very photosensitive seeds, there are other seeds that possess a much lower photosensitivity, although their photorequirement may be equally great. *Chloris ciliata* needs 1-4 hr of daylight in order to show a stimulation of germination (Gassner, 1910). The use of temperature alternations greatly increases the photosensitivity, so that 10 min of daylight is sufficient to stimulate germination (Gassner, 1911a). The minimum amount of light needed to stimulate germination of *Poa nemoralis* is 0.5 hr of daylight (Joensson, 1893). The photoblastic seeds of *Mimulus ringens* need 14 days of sunlight in order to be able to germinate (Hutchings, 1932).

Photosensitivity is a function of temperature, as already mentioned for *C. ciliata*. Whereas for *Lactuca* at 26°C the minimum quantity of light needed to bring about a minimum photoblastic reaction is 0.2 sec  $\times$  250 ft-c, at 30°C about 300 sec  $\times$  250 ft-c is needed to bring about the same minimal effect; i.e., with increasing temperatures the photosensitivity decreases (Evenari, unpublished observation). But for *C. ciliata*

photosensitivity increases with increasing temperatures (Fassbender, 1925).

*Quantity of Light.* Lehmann (1918) and Lehmann and Lakshmana (1924) were the first to point out that for photoblastism the product law (quantity-of-stimulus law) is valid exactly as for photo- and geotropism.

This seems to be true, at least within certain limits. Rao (1925) reports for *Lythrum salicaria* for 3000 MK-sec at 31°C the following germination percentages: 42.8 (10 min  $\times$  5 MK), 37 (1 min  $\times$  50 MK), 37 (30 sec  $\times$  100 MK). For *Lactuca* (Flint, 1934) at 20°C and 3840 ft-c-sec the germination percentages were 53.5 (64 ft-c  $\times$  1 min), 52.8 (32 ft-c  $\times$  2 min), 54.5 (16 ft-c  $\times$  4 min), 58.1 (8 ft-c  $\times$  8 min), 47.5 (4 ft-c  $\times$  16 min), 58.2 (2 ft-c  $\times$  32 min) (var. Arlington Fancy). Similar results for var. Grand Rapids were obtained by Evenari (unpublished observations).

The limitations of this "law" are very great. Here again temperature is an important factor. For *Lythrum* it is valid only for the intensities used between 30° and 35°C. At lower temperatures the intensity range, inside which the product law is valid, is more and more narrowed down. For higher light quantities the effect of light at these temperatures is inversely proportional to the light intensity; i.e., for an equal Meter-Kerzen-second the low intensities used for longer times bring about higher percentages of germination than the high intensities used for shorter times (Lehmann, 1924; Rao, 1925). Exactly the same, i.e., an inverse proportionality of light effect and light intensity when an equal amount of light is used, is reported for some negatively photoblastic seeds, e.g., *Phacelia* (Nicolic, 1924) and three *Bromus* species (Zeiber, 1936), for which the product law is not valid.

When the relation between increasing light quantities and germination for a given light intensity is considered, it stands out very clearly that the curves obtained are logarithmic ones. This was first pointed out by Kincaid (1935) for tobacco seeds and holds true for the figures published by Lehmann and Lakshmana (1924) and by Wieser (1927) for *Lythrum*.

When the seeds are intermittently illuminated, Talbot's law (summation-of-stimulus law) is valid as long as the dark periods between the light flashes are not too long [*L. salicaria*, *Epilobium hirsutum* in Lehmann (1924), Rao (1925), Fassbender (1925)]. If the dark periods are prolonged without a change in the total amount of light applied, the germination is highly stimulated (*ibid*). If this fact can be verified, it seems to us to be of considerable importance in explaining the nature of the light effect upon germination.

Up to now we have considered the effect of different quantities and intensities of light upon germination when light was applied for short periods only. What happens when different light intensities are used in continuous illumination? For *Lactuca* (Evenari, 1952) var. Grand

Rapids at 30°C, germination is an inverse function of light intensity (125, 250, 500 ft-c). The lower the light intensity, the greater the stimulation above the dark germination. At lower temperatures there is no difference between the different light intensities, since maximum germination is obtained with all. The same inverse relation was observed for var. Black Seeded Simpson and var. Oak Leaf at 24°C. For Oak Leaf there was a stimulation of germination at 15, 35, and 75 ft-c (inversely proportional to the intensity of the light) and an inhibition at 130 ft-c of the percentage of germination compared with dark germination.

*Quality of Light.* Cieslar (1883) first reported for the positively photoblastic *Poa pratensis* that yellow light acts like white light, blue light like

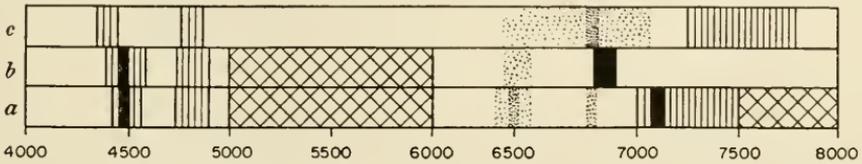
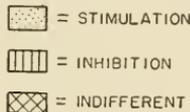


FIG. 11-1. Action spectrum for (a) *Amaranthus* (after Resühr, 1939a); (b) *Phacelia* (after Resühr, 1939a); and (c) *Lactuca* (after Flint and McAlister, 1935, 1937).



darkness. Kinzel (1913-1926) found, in general, that most positively photoblastic seeds were stimulated by light of longer wave length and inhibited by light of shorter wave length. But he also cites some cases in which yellow light stimulates most (e.g., *Veronica peregrina*). Many other authors also found that the effect of red light is like that of white, the effect of blue like that of darkness (e.g., Ernst, 1906; Haack, 1906; Toole, 1938; Eliason and Heit, 1940). Since the most extensive and most accurate work was done with *Phacelia*, *Amaranthus*, and *Lactuca*, we will deal chiefly with these plants. Figure 11-1 gives a comparison of the inhibiting, stimulating, and indifferent wave lengths for these plants, according to Flint and McAlister (1935, 1937) and Resühr (1939a). The most remarkable point is the similarity of the stimulating and inhibiting wave lengths, although *Lactuca* is positively and *Phacelia* and *Amaranthus* negatively photoblastic. This was pointed out for the first time by Meischke (1936) for five positively and eight negatively photoblastic species.

For all three there are two zones of inhibiting light, one around 4400 Å and one around 4800 Å. For *Lactuca* and *Amaranthus* there is another inhibiting region in the near infrared. For *Phacelia* this region is moved to the visible red. There is a stimulating zone for all three around 6400 Å, which for *Lactuca* extends up to 7100 Å. Resühr (1939a) desig-

nates the region between 5000 and 6000 Å as indifferent for *Amaranthus* and as indifferent or slightly stimulating for *Phacelia*. Meischke (1936) reports around 24,000 Å a stimulating zone for positively photoblastic seeds and an indifferent zone for negatively photoblastic seeds. In sharp contrast to these authors, Kommerell (1927) found a direct proportionality at equal energies between percentage of germination and wave length for *Lythrum salicaria* and *Nicotiana tabacum*. In the light of the other authors' research, this does not seem very probable.

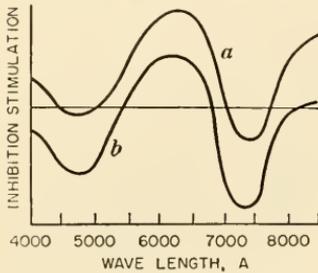


FIG. 11-2. Schematic diagram representing the influence of light of different wave lengths upon the germination of positively (curve *a*) and negatively (curve *b*) photoblastic seeds. With positively photoblastic seeds the stimulating influence is more pronounced than the inhibiting; with negatively photoblastic seeds it is the reverse. Therefore mixed (white) light inhibits germination of negatively photoblastic seeds and stimulates germination of positively photoblastic seeds.

If for a positively photoblastic seed like *Lactuca* and a negatively photoblastic one like *Amaranthus* there are only minor differences between the wave lengths that stimulate or inhibit both, what is the reason for the fact that with one white light stimulates and with the other inhibits germination? The answer is, according to Meischke (1936), to be found in his experiments with differential filters and antagonistic light. These have shown that for positively photoblastic seeds the influence of the stimulating regions is greater than the influence of the inhibiting ones. For negatively photoblastic seeds the reverse is true (Meischke, 1936; see Fig. 11-2).

Another difference between the two types lies in their reaction to indifferent light, which in both cases acts like darkness; i.e., negatively photoblastic seeds germinate well in indifferent light, but positively photoblastic seeds do not (Resüher, 1939a).

We would like to point out here the similarity of the action spectrum for photoblastism and the action spectra of other light-conditioned physiological processes. The action spectra for leaf elongation in etiolated pea seedlings and for floral initiation of barley, cocklebur, and soybean show maxima between 6000 and 7000 Å, i.e., in about the same region where the stimulating-wave-length band for germination is situated; a minimum occurs between 4200 and 5200 Å, i.e., in the same region where our inhibiting sector is to be found (Parker *et al.*, 1949). The maximum in the action spectra of phototropism lies in about the same inhibiting blue region (Burkholder, 1936; Galston, 1950). Whether this is just a similarity or points to a common basic reaction remains to be seen. Certainly research in this direction seems to be promising.

Since only light that is absorbed can act on photochemical processes, there arises the question of the photoreceptors. As the action spectra seem

to be similar, Resühr (1939a) thinks the light-absorbing material inside the seeds must be similar too. Only Flint and McAlister (1937) tried to extract the photoreceptor (or photoreceptors) of *Lactuca* and found good agreement between action spectrum and absorption spectrum of the acetone extract of *Lactuca* seeds, which in turn is nearly identical with the absorption spectrum of chlorophyll. But the authors think that besides chlorophyll there must be another yellow pigment present (perhaps carotenes?) which absorbs the blue inhibiting light. In this connection it is important that Seybold (cited in Resühr, 1939a) found chlorophyll in the fruit coat of *Lactuca* but not in the seed itself, and that Flint and Moreland (1943) are of the opinion that there is evidence of the persistence of chlorophyllous tissue in many seeds.

### 2-3. CHEMICALS

*Interaction of Photoblastism and Certain Chemicals.* Lehmann reported in 1909 that positively photoblastic seeds of *Ranunculus sceleratus* germinated well in darkness when the germination medium was wetted with the nutrient solution of Knop. Gassner (1915b) found that the active part of the nutrient solution was the nitrogen salts it contained, especially potassium nitrate, which since then has been used extensively for stimulating the germination of photoblastic seeds (see Rules for Testing Seeds, 1949). But there are quite a number of positively photoblastic seeds that do not react to potassium nitrate [e.g., *Gentiana andrewsii* (Giersbach, 1937-1938), *Verbascum thapsus* (Gassner, 1915b,c)].

The germination-promoting influence of soil upon *Chloris* (Gassner, 1911b) is explained by the presence of nitrogen compounds in it (Gassner, 1915a; Shuck, 1936). This seems doubtful after Nelson (1927) found that the same nitrogen salts that stimulated the germination of *Poa* when applied in solution inhibited it when added to the soil.

The nitrate effect in bringing about germination of positively photoblastic seeds in darkness is not identical with the light effect, since for *C. ciliata* the light effect is dependent upon temperature, the nitrate effect independent of it (Gassner, 1915a). Leggatt (1946) comes to the same conclusion for *Agrostis* and sums up: "Nitrate while serving in germination tests as partial substitute for light appears to have an entirely different physiological effect." Nitrogen compounds increase the photosensitivity and decrease the photorequirement of *Poa* (Maier, 1933a).

For lettuce seed, thiourea and a number of other compounds containing carbon, nitrogen, and sulfur in a special linkage have the same effect as potassium nitrate (Thompson and Kosar, 1938, 1939). They are also able to break thermodormancy (Thompson and Horn, 1944). Weak acids, too, promote germination of positively photoblastic seeds in certain cases (Lehmann, 1913; Lehmann and Ottenwälder, 1913; Kerbosch, 1920; Gardner, 1921; Fassbender, 1925), but apparently only in combi-

nation with light or alternating temperatures (Lehmann, 1924; Fassbender, 1925).

Hesse (1924) classifies the photoblastic seeds into two groups: .

1. The obligate nitrogen type, where only nitrogen compounds bring about germination in darkness (e.g., *Chloris ciliata*, four species of *Epilobium*).

2. The facultative nitrogen type, where nitrogen compounds and acids promote germination (e.g., *Lythrum salicaria*, two species of *Verbascum*).

It is interesting that, with one exception, negatively photoblastic seeds are not influenced in their germination by acids or nitrogen compounds (Böhmer, 1928).

The following substances, too, stimulate germination of positively photoblastic seeds in the dark: ether, saponin, digitonin (Niethammer, 1928), papayotin, trypsin (Lehmann, 1913; Lehmann and Ottenwälder, 1913). Special mention must be made of coumarin. If nonphotoblastic lettuce seeds are treated with this compound, they are made positively photoblastic (Nutile, 1943-1944, 1945). Many other compounds not chemically related to coumarin and to each other, such as parasorbic acid and sucrose, have the same effect (Weintraub, 1948).

*Naturally Occurring Inhibitors.* A relation exists between naturally occurring inhibitors and the effect of light upon germination (Evenari, 1949). Seed extracts from *Phacelia* inhibit germination to a higher degree in light than in darkness (Magnus, 1920; Peters, 1924; Böhmer, 1928). Extracts of *Pelargonium* leaves (Magnus, 1920) and of *Phacelia* and *Pisum*, for example, have the same effect.

Extracts of seedballs of beet strongly inhibit the germination of beet seed and of a number of nonphotoblastic seeds such as *Avena* (Duym *et al.*, 1947), *Gypsophila*, and *Linum* (Fröschel, 1940) much more in light than in darkness; i.e., they make these light-indifferent seeds negatively photoblastic.

*Gases.* The photoblastic reaction is dependent not only upon pretreatment with different gases (see Sect. 3-2) but also upon the gases present during illumination and germination. It was found for three negatively photoblastic seeds (*Phacelia*, *Nigella*, *Amaranthus*) that with increasing partial pressures of oxygen the inhibition of germination caused by light is greatly reduced (Böhmer, 1928; Resüher, 1939a). Positively photoblastic seeds (*Epilobium*, *Lythrum*, *Nicotiana*, *Eschscholzia*, *Plantago*), when germinated in atmospheres of different oxygen content and illuminated only for some minutes, are inhibited in their germination by increasing oxygen content (*ibid.*).

This inhibition is overcome by higher light intensities (Resüher, 1939a). When continuous illumination is applied, there is no difference in germination for the different oxygen contents (Böhmer, 1928).

The same experiment in darkness gives different results for different

positively photoblastic seeds. *Nicotiana* and *Verbascum* do not germinate in darkness, whatever the oxygen content of the germination atmosphere. With *Daucus*, *Oenothera*, and *Rumex* the dark germination increases with increasing oxygen content (Gardner, 1921). The same is reported for lettuce (Borthwick and Robbins, 1928). Maier (1933b) reports for *Phleum pratense* an increase in the germination in darkness and light alike with increasing oxygen content. When *Phacelia* is germinated under reduced partial pressures of air in darkness, the intact seeds no longer germinate below a vacuum of 220 mm Hg. If the chalaza end of the seed coat is removed, a vacuum of 120 mm Hg is required to prevent germination (Böhmer, 1928).

A very interesting fact was reported for *Lactuca*. If lettuce seeds are germinated at 26°C in darkness in an atmosphere of high carbon dioxide content (5–20 per cent), the seeds germinate up to 90 per cent. Even thermodormancy does not occur at 35°C either in light or darkness when the seeds are exposed to very high carbon dioxide contents [40–80 per cent (Thornton, 1936)]. Leggatt (1948) reports that in an atmosphere of pure carbon dioxide no germination of lettuce seed occurs in light.

### 3. PHOTOBLASTISM AS CONDITIONED BY PRETREATMENT

#### 3-1. PHOTO- AND SKOTODORMANCY

When lettuce seeds in a wet state are retained in complete darkness for several days and then transferred to light, many do not germinate (Shuck, 1936; Nutile, 1943–1944). They entered a “deeper state of dormancy” (Shuck, 1936) or became “skotodormant” (“dunkelhart” of the German authors); i.e., for these seeds light lost its germination-promoting influence.

The same has been reported for many other photoblastic seeds (e.g., Gassner, 1910, 1911a,b; Fassbender, 1925; Maier, 1933a). Negatively photoblastic seeds such as *Phacelia* and *Amaranthus* become photodormant (“lichthart”) through prolonged illumination; i.e., they lose their ability to germinate in darkness (Baar, 1912; Nicolic, 1924; Resühr, 1939a). The intensity of this photodormancy is proportional to the light intensities applied (Nicolic, 1924). In the same way the deepness of the skotodormancy of positively photoblastic seeds is dependent upon the duration of the dark period (Gassner, 1911a,b).

But photo- or skotodormancy cannot be induced in all photoblastic seeds. The negatively photoblastic seeds of *Blitum* and *Celosia*, for instance, germinate well when put into darkness after having been illuminated (Baar, 1912).

Maier (1933b) brought out a very interesting fact for *Phleum pratense*. Whereas the light sensitivity of these positively photoblastic seeds reaches its peak after 12 hr in darkness, it afterward decreases steadily, and after

30–48 hr in the dark the seeds become negatively photoblastic. After 5 days they are completely skotodormant. In lettuce seed, too, when

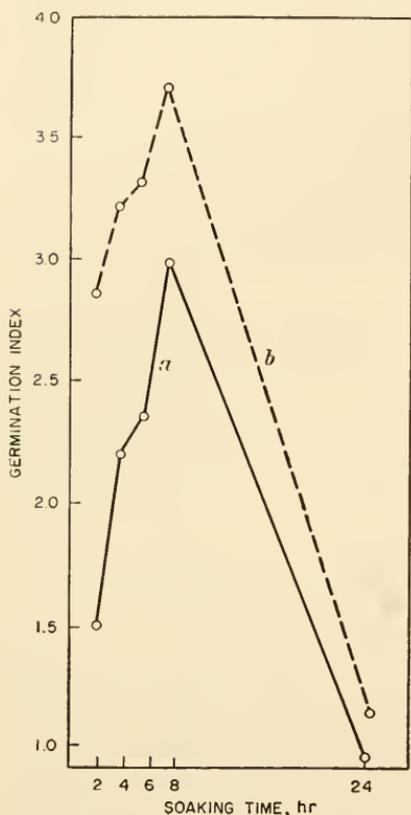


FIG. 11-3. Germination index of Grand Rapids lettuce seed soaked in water in darkness for different lengths of time (2–24 hr), illuminated with 5 sec  $\times$  250 ft-c (curve *a*) and 10 sec  $\times$  250 ft-c (curve *b*) of white light, and then returned to darkness at 20°C. The germination index is the number of seeds germinated after the illumination related to the number of seeds germinated in the dark control, taken as 1.0. The peak of photosensitivity is reached after 8 hr. After 24 hr of darkness the seeds are more or less skotodormant.

photosensitivity remained at the same level. A pretreatment at 10°C brought about a sensibilization that after a duration of 32 days turned into a slight desensibilization. After pretreatments at 22°, 28°, and 40°C, the maximum sensibilization was attained after 2 days. Longer pretreatments cause a strong desensibilization.

darkness is more prolonged than 8 hr, desensibilization sets in until the sensibility is nil and the seeds are skotodormant (Evenari, unpublished work; see Fig. 11-3).

Other seeds (*Epilobium*, *Eschscholzia*) behave similarly, but with tobacco seeds desensibilization, which sets in after about 40 hr of darkness, never reaches the zero point (Bihlmeier, 1927). Desensibilization is influenced by temperature. For tobacco seed it is greatest at 31°C and less pronounced at 28° and 25°C (*ibid.*).

Both positively and negatively photoblastic seeds can be made photodormant by prolonged illumination in blue, germination-inhibiting light (e.g., Eliason and Heit, 1940); this indicates that photo- and skotodormancy are essentially the same phenomenon that is produced when seeds, ready to germinate, are kept for longer periods in conditions unfavorable to germination. This photodormancy can be broken by low-temperature treatment.

### 3-2. PRETREATMENT WITH DIFFERENT TEMPERATURES

Tilly (1934–1935) treated positively photoblastic seeds with different temperatures before giving them a standard illumination. A pretreatment at 3°C caused a pronounced sensibilization, reaching its peak after a pretreatment of 8–16 days. If pretreatment was more prolonged, the

Sensibilization and desensibilization are very much dependent upon the presence of gases during pretreatment. There is no difference between pure oxygen and air. Nitrogen suppresses the desensibilization entirely, but nitrogen and hydrogen stimulate the sensibilization, whereas carbon dioxide retards it.

The effect of temperature pretreatments of short duration (20–60 min) was tried out by Siegel (1950). When *Digitaria* and *Sporobolus* seeds of different water content were exposed to a temperature of 10°C, the seeds became negatively photoblastic. After a pretreatment at 10°–50°C they were indifferent to light. A pretreatment at 50°C and more produced a positive photoblastic response. Brief exposures to 100°C of seeds of flax and radish induced a pronounced negative photoblastic response.

### 3-3. PRESOAKING AND DRYING

Presoaking has a strong effect on photoblastism, since the photosensitivity changes with the time of presoaking preceding the illumination. With *Lythrum*, photosensitivity in certain strains starts after 30 min of presoaking, but with other strains only after some hours (Wieser, 1927). The peak of photosensitivity is reached after about 12 hr for *Lythrum* (*ibid.*) and 16–24 hr for *Eschscholzia* and *Epilobium* (Bihlmeier, 1927). For tobacco seed, according to Kincaid (1935), the time of presoaking needed to initiate photosensitivity is 1 day, and the peak is reached only after about 4 days—a statement that stands in contrast to Bihlmeier's data (1927) for the same plant, indicating much shorter times. For lettuce the photosensitivity is measurable after 4–8 min of presoaking (Evenari, unpublished observations). The curve of its photosensitivity with increasing time of presoaking is given in Fig. 11-4. The existence of a parallelism between photosensitivity, soaking time, and amounts of water taken up was shown for *Phleum pratense* (Maier, 1933b). Temperature is again a modifying factor.

Since light has no effect on dry photoblastic seeds, photosensitivity is clearly a function of the amount of water the seeds imbibe when soaked in water. But the minimum amounts of water needed for the beginning of photosensitivity are different for different seeds. This explains the difference in behavior of different seeds when not presoaked but put in different relative humidities before being illuminated. Tobacco seeds even in a relative humidity of 96 per cent do not become photosensitive as they take up only 20.1 per cent water (Kincaid, 1935). Lettuce seeds, which become photosensitive after only 4–8 min (Evenari, unpublished observations; see Fig. 11-4) of presoaking, develop photosensitivity when taking up 10.1 per cent water from a humid atmosphere (Nutile, 1943–1944).

The light effect is not reversed when, after a sufficient presoaking time, the seeds are illuminated and then dried (Flint, 1934; Kincaid, 1935;

Shuck, 1936); this proves that light, in the presence of a certain minimum amount of imbibition water, initiates certain changes in the seeds which are not reversed when the amount of water needed is removed later on. The same holds true for low-temperature pretreatment of lettuce seed with subsequent drying (Borthwick and Robbins, 1928).

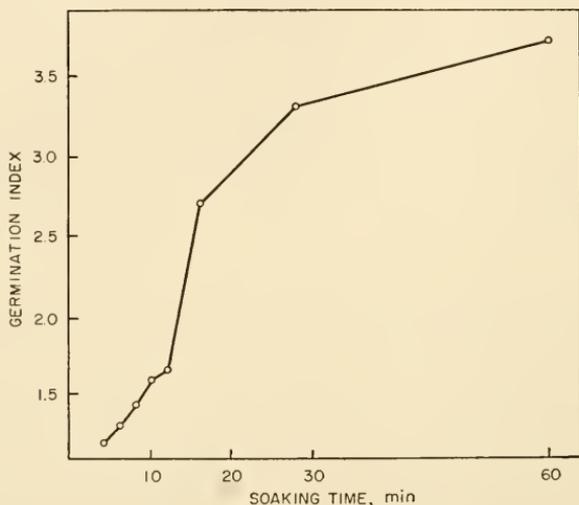


FIG. 11-4. Germination index (see Fig. 11-3) of Grand Rapids lettuce seed soaked in water in darkness for different lengths of time (4-60 min), illuminated with 2 min  $\times$  250 ft-c of white light, and returned to darkness at 20°C.

### 3-4. CLIMATIC CONDITIONS DURING RIPENING OF SEEDS

Since temperature and light pretreatment of the seeds influences the photoblastic reaction, it is not surprising that the climatic conditions under which the seeds ripen when still attached to their mother plants are also important. The seeds of *Drosera* and *Pinguicula* react differently toward light when they are ripened under high- or low-temperature conditions (Kinzel, 1908a). Likewise the grains of *Poa pratensis* react differently toward light when ripened in rainy or sunny weather (Kinzel, 1913-1926). Maier (1933b) reports for *Phleum pratense* a great difference in the photorequirement of different samples. Those ripened quickly during sunny weather have a less pronounced photorequirement than those ripened slowly during a rainy season. Seeds from sunny habitats require less light or lose their photorequirement faster than seeds from less sunny habitats (Niethammer, 1928).

It may be mentioned here that Lehmann (1912) found for *Verbascum* a relation between photoblastism and topographic position of the seed capsules on their mother plant. Seeds from capsules of low position on the inflorescence do not need light for their germination. Seeds from capsules higher up are positively photoblastic.

## 4. PHOTOBLASTISM AS A FUNCTION OF INNER CONDITIONS

## 4-1. INFLUENCE OF COATS ENVELOPING EMBRYOS

Most of the positively or negatively photoblastic seeds germinate well in darkness or light when the fruit or seed coats are removed (Becker, 1913; Magnus, 1920; Gardner, 1921; Hesse, 1924; Bihlmeier, 1927; Böhmer, 1928; Axentieff, 1929; Hutchings, 1932; Resüür, 1939a; Evenari, unpublished work).

Fruit or seed coats are not always the decisive parts, since it was shown for lettuce that only after the removal or puncture of the endosperm was the photorequirement of the seed abolished (Evenari, unpublished work). Very often the whole coat does not need to be removed in order to obtain this effect. It suffices to puncture the seed coat (Lehmann, 1912), to abrade it (Gardner, 1921), or to rub it between the fingers (Resüür, 1939a).

But there are cases of both positively and negatively photoblastic seeds, where the removal of the coats does not affect the photoblastism of the seeds (Axentieff, 1929) or affects it but little (Böhmer, 1928). For the negatively photoblastic seeds of *Phacelia* it has been shown that it is enough to remove the chalaza end of the seed coat to bring about germination in light. The removal of other parts of the seed coat does not affect germination (Magnus, 1920; Böhmer, 1928). Other experiments have shown that the same chalaza end of the seed is the seat of its photosensitivity (*ibid.*; Resüür, 1939a) and that thermodormancy occurs only with intact chalaza ends (Böhmer, 1928). Something similar was observed for the positively photoblastic *Nicotiana*, where the removal of the "Keimdeckel" (i.e., that part of the seed coat which is penetrated by the radicle) stimulates germination in darkness (Bihlmeier, 1927). For many gramineous fruits it has been proved that the hulls that envelop the caryopses are responsible for the photoblastism of the grains, since dehulled grains germinate in light and darkness alike (e.g., Gassner, 1910, 1911a,b; Maier, 1933a,b).

When the dehulled grains of *Chloris*, which are indifferent to light, are wrapped in filter paper, their photoblastism is restored, and they again need light for their germination (Gassner, 1911a,b). The same was observed for decoated lettuce seed (Evenari, unpublished observations). When *Phacelia* seeds that had been made light-indifferent by removal of the seed coat at the chalaza end were covered with filter paper at this part, they were again inhibited by light in their germination (Böhmer, 1928).

Seeds made nonsensitive to white light by decoating are no longer affected by inhibiting blue light (Resüür, 1939a; Leggatt, 1948), and thermodormancy does not occur (Borthwiek and Robbins, 1928; Evenari, unpublished observations for lettuce). It is an important fact that the

coumarin effect on lettuce seed (see Sect. 2-3) also is almost abolished by decoating (Evenari, unpublished observations).

Since the photoblastic influence of light is in some way bound to the coats surrounding the seeds, the light absorption of these coats has been studied. Meischke (1936) found for the seed coats of a number of positively and negatively photoblastic seeds a slow decrease of the light transmission from the red to the violet part of the spectrum [same for *Lactuca* (Evenari, unpublished observations; see Fig. 11-5)]. This is in contrast

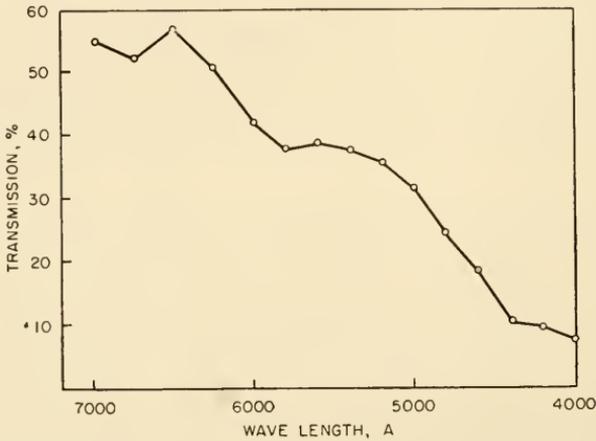


FIG. 11-5. Absorption spectrum of fruit and seed coat of Grand Rapids lettuce seed.

to Kommerell (1927), who found for *Lythrum* and *Nicotiana* a maximum in the absorption curves of the seed coats at the same wave length that produced a minimum in the germination curves.

#### 4-2. AFTERRIPENING

Joensson (1893) was the first to point out clearly that the stage of afterripening of the seed is a decisive factor in photoblastism. Whereas shortly after harvest the seeds of *Poa pratensis* germinated to 88 and 1 per cent in light and darkness, respectively, the germination percentages were 80 and 78 per cent, respectively, 11 months later. Most positively photoblastic seeds behave in the same way. With increasing length of afterripening the photosensitivity increases and the photo-requirement decreases until the seeds become more or less indifferent to light (Laschke, 1907; Gassner, 1911a,b; Reiling, 1912; Kinzel, 1913-1926; Kleine, 1924; Stechmann, 1925; Hite, 1923; Maier, 1933a,b; Thompson, 1935; Shuck, 1936, 1938; Resühr, 1939a). The negatively photoblastic *Phacelia*, too, lost about 50 per cent of its photoblastism after 6 years (Kuhn, 1915). Sometimes the seeds change from positive to negative photoblastism with increasing length of afterripening [Niethammer (1928) for *Stenophragma* and *Bupleurum*].

The photorequirement of immature photoblastic seeds is greater than that of mature ones [Shuck (1934), Thompson (1935) for lettuce]. On the basis of this observation Thompson (1935) tries to explain the fact that the heavy-seeding lettuce variety "Hubbard's Market" is much more photorequiring than the light seeder "Iceberg." In his opinion the varieties with many flowers and seeds cannot provide an adequate supply of nutrients to the seeds, which remain immature to a greater degree.

#### 4-3. PHOTOBLASTISM AND METABOLISM

*Respiration.* The germination experiments with photoblastic seeds in different concentrations of oxygen and the importance of the seed coat for the light effect suggested to early investigators a connection between photoblastism and respiration. Detailed studies have been made on *Nicotiana*. The respiration of *Nicotiana* seeds when germinated in darkness rises with increasing imbibition. The maximum is reached when the imbibition process is completed. Then the respiration curve falls. When the seeds are then illuminated, the decrease in respiration is stopped almost immediately, and shortly afterward the respiration rate rises steeply (Kipp, 1929; Schroepel, 1933).

The respiration quotient remains about constant as long as imbibition continues. Then it falls off, but much more so in the dark than when the seeds are illuminated (Kipp, 1929). After 69 hr in an oxygen-free atmosphere the intramolecular respiration of *Nicotiana* seeds is no longer measurable. When the seeds are then illuminated and allowed to germinate under normal conditions, they germinate as well as if illuminated in air (Wieser, 1927; Schroepel, 1933). This independence of the light effect from the presence of oxygen for *Nicotiana* is in good accord with the germination results. In pure oxygen and in darkness, respiration is more intense than in air and reaches its peak earlier in order to drop more quickly than in air. Illumination brings about a rapid rise, followed by a drop, especially of the carbon dioxide given off, so that the respiration quotient gets smaller and smaller (Schroepel, 1933). This shows that light counteracts the inhibiting effect of pure oxygen for only a short time.

We may mention here a strange observation made by Becquerel (1932) which may be of importance for our problem if sustained by other workers. He found that dry isolated seed coats of *Ricinus* and other leguminous plants show a much stronger gaseous exchange than isolated embryos. When they are illuminated, the gas exchange is strongly stimulated.

Leggatt (1948) investigated the respiration of lettuce seed in blue light, which produces photodormancy. After 7 hr a strong inhibition of respiration sets in. He considers the 7-hr point as a critical stage in the metabolic drift.

*Fat Metabolism.* Gardner reported in 1921 that embryos of various positively photoblastic seeds became more acid when incubated in light than when incubated in darkness [confirmed by Fassbender (1925) and Kummer (1932)]. Since all these seeds contain mainly fat as reserve substance, he concluded that light seems to activate lipolytic enzymes. In grass seeds a relation exists between photoblastism and the content of neutral fats and free fatty acids. Seeds containing large quantities of free fatty acids germinate equally well in light and in darkness. Those containing neutral fats are positively photoblastic (Ralski, 1924). Kummer (1932) reports for different strains of the positively photoblastic *Holcus lanatus* a parallelism between germination and the acid number of fats (see Table 11-1).

TABLE 11-1. RELATION BETWEEN PHOTOBLASTISM AND ACIDITY NUMBER OF FATS FOR DIFFERENT STRAINS OF *Holcus lanatus* (After Kummer, 1932.)

Acid no.	Percentage of Germination	
	In light	In darkness
160	91.5	38.5
74	70.5	1.5
58	59.5	0.5

But light does not appear to influence fat hydrolysis directly, because, in the positively photoblastic *Poa* and in the negatively photoblastic *Bromus*, fat hydrolysis is fastest in light and darkness, respectively. So wherever the germination conditions are best, fat hydrolysis proceeds fastest (Fassbender, 1925; Zeiher, 1936).

*Enzyme Activity.* Early in the history of photoblastic research, different workers were of the opinion that light influences germination by activating or inactivating enzyme systems. As papayotin and trypsin stimulate germination of positively photoblastic seeds in darkness, it was thought that proteolytic enzymes are involved (Lehmann, 1913; Lehmann and Ottenwalder, 1913). Siegel (1950) found that by temperature pre-treatment of *Digitalaria* (see Sect. 3-2) the autolytic activity was significantly increased, whereas the activity of amylolytic enzymes decreased strongly.

There is today no experimental evidence allowing us to relate enzymes and photoblastism, since the rise of catalase and peroxydase activity observed in tobacco seeds after illumination is undoubtedly the consequence of better germination and not its cause (Schroepel, 1933). We may mention here the observation made by Gassner and Franke (1934-1935) that, when positively photoblastic *Lythrum* and *Poa* seeds are made skotodormant, their content of soluble nitrogen remains constant, whereas

they show a decrease of protein nitrogen accompanied by an accumulation of nitrogen in the germination medium. This, according to these authors, indicates that the desensibilization caused by prolonged sojourn in darkness may possibly be connected with the activity of proteolytic enzymes.

## 5. CONCLUSIONS

### 5-1. SUMMARY OF FACTS

Before discussing the theories of photoblastism set forth by different authors, we would like to summarize those facts which are well established:

1. The photoblastic reaction, photosensitivity, and photorequirement are highly dependent upon temperature.

2. The visible spectrum contains bands of stimulating and inhibiting light. The action spectra of positively and negatively photoblastic seeds appear to be very similar.

3. Treatment with different chemicals, foremost among them being potassium nitrate and thiourea, brings about full germination of most positively photoblastic seeds in darkness, abolishing the photorequirement of the treated seeds.

4. Other compounds, like coumarin, bring about positive photoblastism in certain seeds that are normally indifferent to light.

5. The photoblastic reaction depends upon the oxygen and carbon dioxide contents of the surrounding atmosphere.

6. Photoblastic seeds are made dormant by keeping them when soaked for some time under light conditions adverse to their germination.

7. Dry seeds are not photosensitive. Photosensitivity becomes apparent some time after imbibition of water begins, reaches a peak, and then decreases.

8. When photoblastic seeds are exposed to light when wet and then dried, the light effect is preserved.

9. By the removal of fruit coats, seed coats, endosperm, or hulls, photoblastism of many photoblastic seeds is abolished; they then germinate well under otherwise adverse light conditions. After the removal of the coats, embryos do not develop thermo-, skoto-, and photodormancy.

10. With afterripening the photoblastism of most photoblastic seeds changes. Generally photorequirement decreases and photosensitivity increases with increasing afterripening.

### 5-2. THEORIES

As point of departure we take Gassner's theory (1911a,b) of photoblastism, which he developed in his basic research on *Chloris ciliata*. The hulls render the entry of oxygen into the germinating grain more difficult. This germination inhibition is counteracted by light; i.e., the hulled grains

are positively photoblastic. Dehulled grains do not require light for their germination, since the free entry of oxygen is ensured by the absence of the hulls. Dehulled grains are made positively photoblastic by insufficient oxygen supply, nonoptimal temperatures, and insufficient after-ripening. All three factors cause, in Gassner's opinion, a decrease of the germination energy, i.e., a retardation of germination. During the germination of *Chloris* two different processes occur side by side: one process typical for the germination of all seeds, photoblastic and nonphotoblastic alike, and another one typical for photoblastic seeds only. This process, according to Gassner, consists in the formation of an "inhibiting layer" or "inhibiting principle" inside the fruit or seed coat. This inhibiting layer does not exist beforehand but is formed during the initial stage of germination. In darkness there is nothing to counteract the formation of this layer, so that there is no germination in the dark. The desensibilization processes, leading to skotodormancy, are, according to Tilly (1934-1935), an indication of the formation of this inhibitory layer, which is destroyed by nitrates, acids, and other chemicals (Hesse, 1924). Light inhibits or retards the formation of this layer; i.e., light acts on the coats, inside which certain chemical processes bring about the formation of the inhibiting layer. Insufficient oxygen supply, nonoptimal temperatures, or insufficient afterripening inhibits germination in the dark as a result of a decrease in the germination energy and the formation of the inhibiting layer before the radicle can penetrate the seed coat. When light is present, this retardation of germination does not matter, since the light inhibits or retards the formation of the inhibiting layer.

The first important point in this theory is the role played by the hull or the fruit and seed coats. Most authors agree that the coats interfere with the gas exchange necessary for normal germination. There can be no doubt that the coats deeply modify the nature of seed respiration (e.g., Frietinger, 1927). This explains why the removal of the coats brings about germination under conditions that do not allow germination of the intact seed. But this inhibition of normal gas exchange may be an inhibition of oxygen entry into the seed (Gassner, 1911a,b; Becker, 1913; Davis, 1924; Axentieff, 1929), or it may be an inhibition of carbon dioxide exit from the seed into the atmosphere (Leggatt, 1948). The inability of the intact seeds to germinate would be explained in the first case by the lack of the necessary oxygen and in the second case by the presence of carbon dioxide, which produces dormancy (*ibid.*). We may add here that Forward (1949) found for oats that carbon dioxide brings about dormancy and that the dormant condition of freshly harvested grains is caused by accumulation of carbon dioxide. This dormancy is broken by any method that either sets the carbon dioxide free (pricking of coats) or increases the concentration of a substance (oxygen) that counteracts the carbon dioxide effect.

Another important point is Gassner's opinion that the light effect is localized in the coat. This idea is strongly contested by Lehmann and his school, who believe that the inner living part of the seed is affected by light, and not the coat.

According to Lehmann (1913), for example, light acts catalytically upon the living seed content. He bases this opinion first of all on the fact that the laws of stimuli are valid for photoblastism and that these laws apply only to living matter. Crocker (1929) remarks very rightly that the product law is valid only in very narrow limits and that it is possible that within such limits it could apply to nonliving tissues as well as to living matter. It may be added that it is valid even for the photographic plate.

But there are other facts showing that the light effect may be in part upon the embryo and not the coat. Only two will be cited. Leggatt (1948) produced photodormancy in lettuce by exposing the seeds to blue light. He then dried the ungerminated seeds, pricked the coats of some, and germinated pricked and unpricked seeds in light and darkness. In light, pricked and unpricked seeds germinated equally well; in the dark, unpricked seeds had a very low germination percentage, and the germination of the pricked seeds was retarded. If the blue light effect had been primarily upon the coat, equal germination of the pricked seed in light and darkness would have been expected. Bihlmeier (1927) reports for *Nicotiana* that those decoated seeds which did not germinate in darkness germinated to about 30 per cent when exposed to light.

But, wherever the light effect may be localized, what is its nature? Gassner leaves this question more or less open. Most authors describe the light effect as "photochemical." This opinion is supported by the observation of Kincaid (1935) that with *Nicotiana* the light effect is independent of the temperature at the time of the exposure to light. Gardner (1921) is of the opinion that light activates lipase in the seed coats of *Rumex crispus*. The enzyme then hydrolyzes the lipoids of the coat, thus making them more permeable. But most authors think that the light effect is in some way related to oxidation-reduction phenomena (Axentieff, 1929; Böhmer, 1928; Kipp, 1929; Leggatt, 1948). Light stimulates or inhibits oxidation processes during germination (Axentieff, 1929). As for positively photoblastic seeds, the oxygen optimum for germination lies around 20 per cent. Böhmer (1928) thinks that, up to this optimum, light and increasing oxygen content stimulate germination, since the oxygen taken up is needed for respiration. When the oxygen content is further increased, the oxygen taken up is not used for respiratory processes and starts processes antagonistic to the light effect. Then increasing amounts of light are needed to counteract the inhibiting effect of rising amounts of oxygen. For negatively photoblastic seeds the order is reversed: oxygen above the amount needed for respiration

starts processes that bring about germination, and light counteracts this (*ibid.*). Kipp (1929) elaborated upon this idea. In the seed there is an equilibrium oxidation-reduction reaction, which is changed by light and different oxygen concentrations. Light pushes the equilibrium toward reduction; oxygen, above the amount needed for respiration toward oxidation. An enzyme that is indispensable for germination is reduced and so activated or oxidized and inactivated. Negatively photoblastic seeds can germinate only when the equilibrium is changed toward oxidation.

Leggatt (1948) is of the opinion that the inhibitor of germination is carbon dioxide, which cannot escape from the seeds so long as the coats are impermeable to it. He thinks that under the influence of carbon dioxide a type of zymase appears which produces little alcohol and much acetaldehyde, which, by the way, was earlier considered by Mazé (see Evenari, 1949) as a germination inhibitor. This brings us to the question of photodormancy, since Leggatt (1948) found that, when lettuce seeds are made photodormant by illumination with blue inhibiting light, they contain less alcohol than seeds illuminated with white light. Accordingly he thinks that photodormancy is brought about in the same way as the germination inhibition caused by carbon dioxide. It may be mentioned that Wieser (1927) had tried to explain skotodormancy by supposing that, when seeds are put to germinate in darkness, germination starts. But, owing to lack of oxygen, intramolecular respiration sets in. Substances are produced which inhibit germination and make the seed unable to react later to the photochemical effect produced by light.

Bünning (1948) thinks that light starts two diametrically opposed processes in germinating seeds: one inhibiting and one stimulating. This opinion is strongly supported by the fact that there are inhibiting and stimulating wave-length zones that are nearly identical in position and effect on positively and negatively photoblastic seeds. Possibly there are even two different photoreceptors, carotene for the inhibiting and chlorophyll for the stimulating wave lengths. It is of interest that there seems to be a similar dual effect of light upon stomatal movement, one set in motion by blue light (absorbed by carotene?) and one brought about by red light (absorbed in chlorophyll?) (*ibid.*, p. 361). In connection with this we may mention that Flint and Moreland (1943) found for *Hymenocallis occidentalis*, whose integuments contain macroscopically visible chlorophyll, dependence of germination upon the photosynthetic activity of the integuments. This, in their opinion, explains the observation that in a carbon dioxide atmosphere the seeds germinated well in light but did not germinate in darkness.

A point difficult to explain is the fact that photoblastism is a function of temperature. Gassner (1911a,b), for example, believes that at low temperatures light inhibits the germination of *Chloris*, which is positively

photoblastic at higher temperatures, because light, besides retarding the formation of the inhibiting layer, decreases the germination energy at low temperatures. There are no facts to support his opinion.

A better explanation is given in those cases in which low temperature makes seed that are positively photoblastic at higher temperatures independent of light (lettuce seed, for example). For these seeds Davis (1924) has pointed out that low temperature decreases the oxygen requirement of the seeds, which can then germinate in the dark with the restricted oxygen supply that passes through the coats. There is naturally a further possibility, i.e., that low temperatures increase the permeability of the coats toward oxygen or carbon dioxide, and it may be that both effects of low temperatures work together. We mention in this connection the findings of Brown (1940) that the permeability of *Cucurbita* seed-coat membranes toward carbon dioxide was different at different temperatures.

An entirely different approach to the temperature effect, worthy of close investigation, was given by Bünning (1948). He explains the different effects of light at different temperatures by supposing that the stimulating and inhibiting processes produced by light are dependent upon temperatures to varying extents.

The fact that with afterripening photorequirement decreases and photosensitivity increases could be explained by supposing that afterripening increases the permeability of the coats toward oxygen and decreases the oxygen requirements of the embryo. This was pointed out by Shull (1909, 1914) for *Xanthium*. Or it may be that by afterripening the relative amounts of inhibiting and stimulating photoreceptors are changed (Bünning, 1948).

In reviewing all these different opinions brought forward to explain photoblastism, we can only agree with Crocker (1936), who states that the factual evidence for any one of these theories is entirely inadequate. One of the reasons for this, besides the main one, i.e., the extremely complex nature of the processes involved, is to be found in a fact pointed out by Resühr (1939b). When we speak about germination, we mean by this the penetration of the seed coat by the radicle. At least three different seed parts have to join forces in order to bring about this process: embryo, seed coat, and endosperm. If a seed does not germinate, we do not know which part is responsible, since each one alone can be the limiting factor. When we obtain the same percentage of germination, it may be brought about in each case by a completely different relation of the three factors involved. Without deeper knowledge of this, we would jump to the conclusion that the obtaining of the same percentage of germination in different experiments is due to the same factor—a conclusion that may not be true.

## 5-3. SUGGESTIONS FOR FURTHER WORK

Summing up, we would like to suggest the following lines of approach for future research on photoblastism:

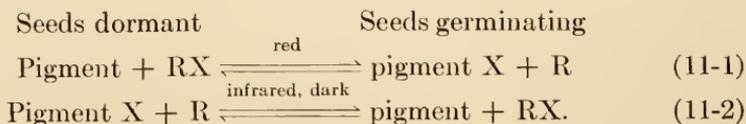
1. Isolation and identification of the photoreceptor or receptors involved.
2. Research into the enzyme systems involved, with special stress upon photoactivation or inactivation of enzymes along the lines worked out by Galston (1950) and Galston and Baker (1949).
3. Research into the changes of permeability of artificial membranes brought about by light [see Brauner and Brauner's studies (1940) on the photoeffect of membrane permeability].
4. Clarification of the relation between respiration, light, and special inhibitors like coumarin.
5. Research into the specific role played by coats, endosperm, and embryo in normal germination, dormancy, and photoblastism.
6. Research into the mechanism of the interrelation between temperature and photoblastic reaction.

## 6. ADDENDUM

After this manuscript was ready for publication, some new contributions to the problem of photoblastism were made. Poljakoff-Mayber (1951, 1952) tried to elucidate the influence of light and coumarin on the metabolism of germinating lettuce seeds. Evenari (1952) investigated the interaction of light, temperature, and coumarin on the germination of different varieties of lettuce seeds.

It could be shown that under certain conditions all varieties are photosensitive, even those whose germination generally would be considered independent of light. The interaction of the three factors investigated is explained by the change of thermosensitivity caused by light and treatment with coumarin.

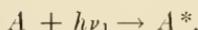
Borthwick and coworkers (1952) proved that the germination of lettuce seeds stimulated by red light can be inhibited by infrared radiation and that this inhibition again can be suppressed by red light, and so on. This brings the authors to the following scheme of light action:



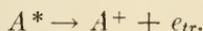
This means that in this reversible photoreaction two pigments are involved as well as other reactants. The percentage of germination under given conditions depends upon the integrated absorption of light by the two pigments.

It is most important that the light effects on the germination of lettuce seeds and photoperiodic responses seem to be identical.

Evenari and Stein (1953) proposed a somewhat different theory of photoblastism. The fundamental process involved in the induction of germination consists in an electron transfer from a dye  $A$  with an absorption peak at about 6600 Å:



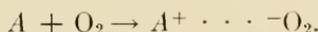
An excited molecule  $A^*$  is formed which may lose its excited electron to a neighboring trap:



The absorption peak of the new system shifts to approximately 7300 Å. The absorption of a quantum of suitable energy will bring about the release of the trapped electron,



An increase in temperature may also supply the energy of activation for liberating the electron from the potential well. The entire metastable system may be one belonging to the original absorbing molecule. Oxygen would react with the original dye with total or partial electron transfer,



According to this scheme, the odd ion  $A^+$  is of paramount importance for germination, since it would initiate germination either in that form or through its associated free radical  $B$ , where



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## Viscosity, Permeability, and Protoplasmic Streaming

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*Influence of visible light on viscosity of protoplasm. Influence of visible light on permeability of cells: Indirect influences—Direct influences. Influence of visible light on protoplasmic streaming. References.*

The effects of visible light on the viscosity, permeability, and streaming of the protoplasm can presumably be interpreted as intermediate links in a chain of reactions induced by light or as secondary effects of such reactions. It is probable that this chain of reactions includes changes in the structure of the protoplasm. There are many indications that this is the case. If this assumption is correct, it may be presumed that the structural changes are the primary manifestations and that the changes in viscosity, permeability, and streaming of the protoplasm are secondary to them. This would, however, also imply that—as long as the knowledge of the structure of the protoplasm is incomplete or hypothetical—the most important premises are lacking for an analysis of the relation between these secondary phenomena and the influence of light.

### INFLUENCE OF VISIBLE LIGHT ON VISCOSITY IN PROTOPLASM

The degree of viscosity and its variations are determined by the composition and structure of the protoplasm and the variations occurring in it. Our interpretation of the cause of viscosity and of its nature is mainly based on the conception of the plasmatic structure. If the protoplasm is envisaged only as a system of dissolved and dispersed substances, the viscosity can be explained as a result of the friction between the particles in this system and as being of the same nature as the viscosity in solutions and colloids in general. Staudinger (1935, 1947) classified the colloids according to the shape of the particles into spherical colloids, which are composed of spherical particles and whose viscosity is relatively low, and thread colloids, consisting of elongated particles with a high viscosity. In both categories the viscosity is dependent on the concentration, temperature, degree of dispersion, solvation, electric charge, and

the preliminary thermal and mechanical treatment of the substances. It is also dependent on the substances administered and on time. Moreover, in thread colloids the viscosity increases with the length of the chain molecules. Because protoplasm contains both spherical and thread colloids, its viscosity is dependent on all these properties.

If, on the other hand, the view held by Seifriz (1936, 1942), Frey-Wyssling (1948), and others is adopted, i.e., that the protoplasm not only is an amorphous system of colloids and solutions but also possesses an internal structure, additional factors will affect the viscosity. According to this conception, the principal components of the protoplasm, the protein molecules, are either joined into a framework by means of their side chains or first joined into bundles (micelles). The latter in turn build up the framework, to which enzymes, lipids—including phosphatides—inorganic groups, water dipoles, etc., may be attached. The spaces in the framework—the intermicellar spaces—are filled with water and with substances dissolved and dispersed in it. These interpretations have not been refuted by the observations of the structure of the protoplasm hitherto made with the electron microscope (e.g., Mudd, 1947).

Local concentrations in the framework may cause the occurrence of microscopically visible fibrils and other corpuscles. Corresponding local dilatations in the interstitial spaces can give rise to vacuoles and to droplets. The fibrillar and the alveolar hypotheses were based on the occurrence of such morphological differentiations.

If this hypothesis for the plasmatic structure is accepted, it implies that the viscosity of the protoplasm is conditioned not only by the viscosity of the interstitial fluid but also by the plasticity and elasticity of the framework.

It can be assumed, on the foregoing premises, that the viscosity of the protoplasm is a more complicated phenomenon than the viscosity of solutions and colloids in general. The viscosity of the protoplasm will then be the aggregate of the viscosity in its solutions and colloids (the dispersion viscosity) and of that caused by the junctions of the elements in the framework (the structural viscosity). Frey-Wyssling (1948) assumed that the protein molecules and the other components of the plasmatic framework are united by different kinds of valence bonds and cohesive bonds. He also assumed that the framework can be successively or partially dissolved and reunited by means of the breaking and joining, respectively, of these bonds. Consequently, the physical properties of the protoplasm (fluidity, plasticity, and elasticity) may be attributed to the characters of the various junctions. The more these are dissolved, the more liquid the protoplasm becomes; i.e., its viscosity decreases. Thus, because the structural viscosity varies with the joining and breaking of the bonds and the dispersion viscosity is constantly affected by the changes in the factors enumerated in the foregoing, the viscosity of the

plasma is labile. Moreover, it varies with the physiological condition and development of the organism (see Seifriz, 1936).

Weber (1929a) was presumably the first to study the effect of light on the viscosity of the protoplasm. He infiltrated leaves of *Ranunculus ficaria* with a strongly hypertonic solution of potassium chloride and calcium chloride and observed the course of plasmolysis in the cells of the spongy mesophyll. He compared leaves exposed to sunlight under natural conditions or to diffuse daylight with leaves that had been kept in the dark. In the last-mentioned, plasmolysis took place rapidly, and the protoplasm assumed a convex form. He interpreted this as a sign of low viscosity. In the leaves exposed to light, on the other hand, plasmolysis was retarded, and the protoplasm freed itself from the cell wall with concave surfaces. These results give the impression that the protoplasm has a high viscosity (adhesion, stickiness). In another study Weber (1929b) demonstrated that, as a result of such differences, the onset of plasmolysis may vary from a few seconds to a long period. Weber did not, however, make any definite statements regarding the mechanism of the effect of light. He left as an open question whether light has a direct effect on the protoplasm or whether its action takes place as a result of other effects, such as heating, transpiration, or photosynthesis.

By means of centrifugation of the objects, Alsup (1942) studied the effect of light on the viscosity of the protoplasm in *Amoeba proteus* and *A. dubia* and the dependence of the effect on the presence of calcium. Centrifugation was performed with a hand centrifuge, and the moving of the granules was observed. He used a microscope lamp of approximately 300,000 m-c as the source of light. The experiments showed that illumination increased the viscosity of the cortical protoplasm ("plasmagel") approximately 30 per cent and that this change started immediately. The increase in viscosity could be detected within 10 sec after the exposure and persisted for over 15 min. Calcium was found to be essential for this gelating action, since light did not increase the viscosity of the plasmagel of amoebae immersed in ammonium oxalate. The "plasmagel," or inner protoplasm, of *A. dubia* was also gelled by short exposures to the intensive light, and the effect persisted for at least 3 min. It could also be prevented by removal of calcium from the cells with oxalate.

In investigations made by Stålfelt (1945, 1946), leaves of *Elodea densa* served as the test object, and the viscosity was measured by centrifuging the leaves. The centrifuge (angle type, about 3000 rpm) had four tubes, and eight leaves could be tested at the same time. On centrifugation the chloroplasts moved to the distal end of the cells. The time required for this moving was taken as an indicator of the viscosity of the protoplasm. After centrifugation the chloroplasts returned to their normal positions, and streaming continued as prior to it. No detrimental influ-

ence of centrifugation could be observed. According to Beams (1949), the majority of the *Elodea* cells survive an ultracentrifugation of even 350,000 times gravity for 30 min.

Only the apical part (20–30 cm at the top) of the shoot was used in the experiments. The time required for the chloroplast movements was found to be the same for all the leaves of the shoot at the same time, but the value changed from hour to hour. When, however, the material was kept in the dark for 2–3 days, the fluctuations ceased, and the time of centrifugation was constant or almost constant from hour to hour and during a shorter period from day to day.

Objects subjected to such pretreatment were examined in the light; ordinary electric-light bulbs were used as the source of light, which passed through two glass cuvettes with running water to a depth of 15 cm.

The plasmatic equilibrium, gradually reached in darkness, was immediately upset when the leaves were exposed to light of 5–48,000 m-c intensity. After as short a time as 15 min, the first effect was noticeable. Subsequent increases and decreases in the mobility of the chloroplasts followed after varying periods. An exception to this rule was noted in the experiments with strong light (32,000 and 48,000 m-c). During the first few minutes of illumination the time of centrifugation dropped to a low value that became nearly constant during the following hours. This means that strong light has a paralyzing influence upon the reactions due to the viscosity.

Many changes in the state of the protoplasm were also found when the light was limited to the first hours or minutes, thus indicating the occurrence of aftereffects. Illumination of 100 m-c for 15 sec sufficed to induce the alterations, which persisted for several hours.

Owing to the aftereffects of light, the plasmatic system never reaches photic equilibrium under natural conditions. In the morning and during the day the leaves are exposed to fresh light inductions, whose effects do not become exhausted during the following night. Centrifugation values with hourly variations are always found when test objects are taken from plants growing under natural conditions or in ponds. Weber (1925a,b) also found that the viscosity of the objects studied by him (*Elodea*, *Spirogyra*, succulents) varied in the course of the day and with the seasons.

The photic effect seems to be strictly localized to the illuminated part of the object and even to a part of a cell (see Fig. 12-1).

Virgin (1948) made centrifugation experiments, but with a somewhat different method. Instead of using slides, he inserted the *Elodea* leaves in small glass tubes, thus reducing the pressure on the cell wall to a minimum. His experiments showed that the changes in the protoplasm caused by light occurred in more regular periods if the plants were allowed to grow in a constant light and temperature (e.g., 450 m-c and 20°C) for

some time before the experiment. An intensity of light of 0.5 m-c sufficed to cause variations.

Virgin (1947, 1948) also found the same reactions in *Spirogyra* and *Mnium*. In *Spirogyra*, however, the time of centrifugation increased when the objects were kept in the dark. It rose from 2 to 15 min in the course of 4 days. There was a rapid decrease when the leaves were exposed to light, and after about 3 hr the time dropped to 2 min.

All the test objects studied, i.e., *Elodea*, *Spirogyra*, and *Mnium*, showed that visible light causes changes in the viscosity of the protoplasm. It is

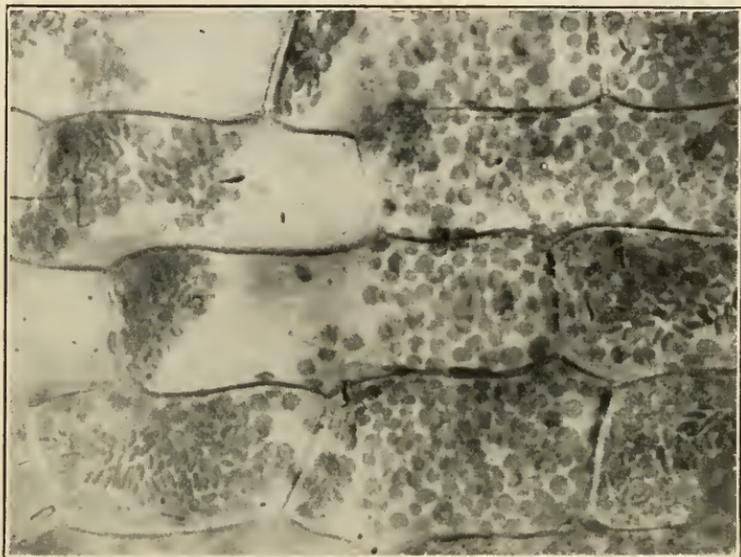


FIG. 12-1. Centrifuged leaf cells of *Elodea densa*. One-half the area has been illuminated, and the other half kept in darkness (covered with tin foil). In the illuminated cells, and even in parts of the cells, the chloroplasts are moving and accumulating in the distal end. (Courtesy of H. Virgin, 1950.)

therefore possible to assume, as did Virgin (1948), that the phenomenon is a general one and that it occurs presumably in all green plants. Difficulties have nevertheless been encountered in confirming the accuracy of this hypothesis. This is because the centrifugation method can be used only when the test object fulfills certain criteria, so that only a few species are suitable. Other methods, such as the plasmolytic, the gravity, and the Brownian movement, either have too slow an action or record movements of the particles that can be simultaneously affected by the microscope light, since this has an effect on the state of the protoplasm. Moreover, the light emanated by the microscope is so strong that the viscosity of the protoplasm rapidly reaches the paralytic stage. Owing to its constancy and lack of sensitivity, this can easily give the impression that the viscosity is unaffected by light.

In these experiments the influence of light on the time of centrifugation has been interpreted as due to its effect on the resistance of the protoplasm through which the chloroplasts move, i.e., on the viscosity of the protoplasm. It cannot be connected with photosynthetic processes, because the plasmatic changes are induced by light quantities insufficient to reach the compensation point of photosynthesis, and of such short duration that the photosynthetic production would be practically nonexistent.

The mechanism of the influence of light is as yet unknown, but there are two possible explanations:

1. If the dispersion viscosity is involved, it is conceivable that light influences the electric charge of the particles, thus affecting their hydration and thereby their volume and friction. Support is lent to this hypothesis by the investigations of L. Brauner (1935, 1937). He found that the charge of membranes is changed by light and that this change has a rapid onset. Pincussen (1930), who made experiments with acidoid gels, stated that light caused a reduction in the charge and a slight shrinking of the gel. Pincussen also recalled Young's studies (1922), which showed that the greater the amount of protein solutions freed from the electrolytes, the greater is their sensitivity even for long-wave-length light. Pyrkosch (1936) and others (see Lepeschkin, 1938, p. 192) later obtained similar results. Pyrkosch found that both the light from a 200-watt bulb and monochromatic light caused a change in the colloidal state of milk. Ultraviolet light, however, had a considerably more marked effect.

The same similarity in the effect of visible light and ultraviolet light appears in the case of the viscosity. In visible light the effects are reversible and form part of the normal processes occurring in the protoplasm, but ultraviolet light—at any rate, in large doses—has a destructive effect in that concentration continues and coagulation occurs (Gibbs, 1926; Heilbrunn and Young, 1930). The similarity between the influences of the respective kinds of light indicates that the same processes or states are affected and that the difference in the manner of action is only of a quantitative nature. In long-wave-length light the changes are less marked or only moderate and therefore reversible, whereas in short-wave-length light they are intensified and exceed the border line to irreversible changes. As will be shown in the following, the same difference between the two kinds of light is also noticeable in their effect on the permeability.

2. On the other hand, if the structural viscosity is altered, it might be supposed that light exerts its influence through the mechanism of the bonds by which the elements of the plasmatic framework are joined. The more the bonds are broken, the more fluid will the protoplasm become.

It is also conceivable that more special processes are involved in the mechanism. As mentioned earlier, Alsup (1942) found that calcium is essential for the action of light. He therefore made an attempt to bring

his observations into accord with Heilbrunn's calcium-release theory (1937). According to this theory, the stimulating agents—among them light—cause a breakdown in a cortical calcium-containing gel, with the resultant passage of free calcium ions to the cell interior. It causes a brief transitory liquefaction at this site, soon followed by gelation or clotting of the protoplasm. Alsup stated that calcium must be associated with the influence of light but that experimental results do not otherwise substantiate the theory.

## INFLUENCE OF VISIBLE LIGHT ON PERMEABILITY OF CELLS

### INDIRECT INFLUENCES

The relation between light and permeability is complicated, particularly in the higher plants, by the fact that the exchange of water and solutes in cells and organs is dependent on the physiological state of other parts of the same plant and on the physiological processes taking place there. Light may exert either an indirect influence on the exchange of substances by affecting such states and processes or a more direct influence by affecting the permeability of the cells. This twofold effect constitutes an unfavorable factor for the analysis of the influence of light on the permeability. Because both modes of action are intermingled physiologically, it is often difficult to determine which one causes an observed alteration in the light-induced exchange of substances.

That indirect effects occur has been shown by the following experiments:

Schmidt (1936) found that illumination of the aerial parts of higher plants changed the salt intake of the roots growing in the dark. As a test object he used *Sanchezia nobilis*, which he grew in a nutrient solution. Since the intake of ions is dependent on, among other factors, the quantitative intake of water, the experiments were arranged in such a way that the water metabolism of the plants was uniform. Illumination (2500 m-c) of the aerial shoots was accompanied by an increase in the intake of potassium, magnesium, and the nitrate ion and a decrease in the intake of calcium and phosphate by the roots growing in the dark. The effects varied with the strength of illumination. With an increase in the intensity of illumination of the shoots, there was an increase in the intake of potassium and magnesium by the roots. A maximum was reached at approximately 2500 m-c and remained constant with an increase in the strength of illumination. Conversely, a decrease occurred in the intake of nitrate, phosphate, and calcium, a minimum being reached at approximately 900–1000 m-c. The intake subsequently rose with the increase in the strength of illumination. Within the minimum range the roots gave off calcium and phosphate to the surrounding solution. The salt intake of the roots was also found to be dependent on the pretreatment of the shoot with light or darkness.

Alberda (1948) cultivated *Zea mays* in nutrient solutions of different concentrations. In darkness the absorption came to a standstill with the "high-salt" plants (grown in solutions of higher concentrations) and, in most cases, even passed into an exit of ions. "Low-salt plants" (grown in more dilute solutions) were, on the other hand, capable of absorbing phosphate even in darkness.

Alberda concluded from his experiments that the growth of the shoot is determinative for the absorption of the high-salt plants. He explained this correlation between shoot and root by the hypothesis that, with high-salt plants, a part of the ions secreted by the root in the xylem are carried back to the root by the phloem when the consumption by the shoot is less than the supply by the root.

Investigations of Andel *et al.* (1950) have shown that the influence of light on the intake of salts can be altered by changing the balance between the sugar production and salt content of the plant. They cultivated maize in light and in darkness, glucose being administered to the roots in some experiments. They stated that the intake of phosphate increased when glucose was added to the nutrient solution. This connection between sugar and phosphorus was explained by the authors as an acceleration by sugar of the synthetic processes by which phosphorus is fixated. Glucose also increased the intake of phosphate by plants continuously exposed to light, but the intake diminished when, after a long dark period with glucose, plants were once more placed in the light, but without glucose. From this fact it was concluded that under normal conditions the sugar content of the root is too low to obtain a maximal absorption of phosphate.

It was also found that the intake of potassium was to a great extent dependent on the exposure to light, but that it was not furthered to any considerable degree by the addition of sugar to the nutrient solution. In dark there was a distinct exit of potassium, but not of phosphate, from the roots to the solution. In accordance with Alberda (1948), the authors explained this exit of potassium as a consequence of the retarded growth of the shoot in the dark. In these experiments the effect of light on the exchange of solutes could be explained without the necessity of assuming any change in the permeability. No proof that this had not actually occurred was forthcoming, however, and therefore the possibility of a photic effect of this nature cannot be ruled out.

#### DIRECT INFLUENCES

In a number of other studies the experiments were arranged in such a way that the light had a more direct effect on the permeability of the cells.

Lepeschkin (1908a,b, 1909) was the first to report experiments of this kind. He studied the movements of the leaves of *Phaseolus*, *Mimosa*,

and *Desmodium*. His results could be interpreted on the basis of the assumption that the changes in light gave rise to changes in the permeability of the plasmatic membranes. These changes in permeability would, in turn, lead to changes in turgor and the known turgor movements of the leaves. Lepeschkin extended his experiments to other test objects such as *Spirogyra* and the epidermal cells of *Rhoeo*. Since he found similar changes in permeability, he considered that the sensitivity of the permeability to light is not specific to the cells of the pulvini but that it is a general phenomenon.

In his first experiments Lepeschkin used three different methods to demonstrate the effects of light upon the permeability of protoplasm. The first comprised a determination of the amount of substances diffusing into water from the pulvini. The concentration of cell sap in slices immersed in water was observed with the second method. The most important method was that of the isotonic coefficients, since this was subsequently used by many other investigators who worked on the problems of permeability.

*Experiments with Plasmolytic Methods.* Lepeschkin compared the experimentally found isotonic coefficient for the plasmolytic medium with that calculated theoretically. He considered that, if the coefficient found was greater than that calculated, it would imply that the plasmolytic substance had penetrated into the cell. The difference between the coefficients would then be a measure of the permeability of the cell to the substance in question. Lepeschkin found this difference to be accentuated by light.

At approximately the same time, Tröndle (1910) reported experiments that substantiated the accuracy of Lepeschkin's conclusions. In Tröndle's experiments, made on leaves of *Buxus* and *Tilia* and carried out with the method of coefficients, an association was also found between the permeability (to sodium chloride) and light. In the dark, permeability was very slight; it increased with a certain intensity of light and decreased with still stronger light, this decrease continuing with even more powerful illumination. He also found that the illumination with an electric lamp of 32 cp at a distance of 10 cm from the plant first resulted in an increase in permeability, followed by a decrease if the illumination continued for more than 1 hr. In a later publication (1918) Tröndle repeated his earlier experiments and also tested another method. This was based on the determination of the quantity of salts taken up by the test object per unit of time. The results of these experiments supported his earlier conclusions.

Fitting (1915, 1917, 1920), who made a thorough study of the plasmolytic method, introduced several improvements. Instead of the isotonic coefficients, he made use of deplasmolysis. Provided that deplasmolysis takes place owing to penetration of the plasmolytic substance into the

cell, its rate would be an indicator of the rate of permeation, i.e., of the permeability. Fitting (1915) found a change in permeability in the course of the experiments, it being possible to record changes within 15 min. He therefore concluded that the plasmolytic substance either affected the plasmatic membranes, causing a change in their properties, or gave rise to changes in the content of osmotically active substances in the cells. Because these processes affect the values of the isotonic coefficients, he criticized the method used by Lepeschkin and by Tröndle. He also raised the objection that these authors had not taken into consideration the possibility of an exit of osmotically active substances from the cells. Lepeschkin (1923) countered this objection by stating that such deficiencies are common to all plasmolytic methods and were therefore also applicable to those used by Fitting. Moreover, as pointed out by Tröndle (1918), Fitting used a relatively unsuitable test object, *Tradescantia*. The permeability of the leaves of this plant is so slight that the experimental errors will be relatively large in proportion to the results.

Zycha (1928), using the same test object as did Lepeschkin and Tröndle, i.e., *Rhoeo* and *Buxus*, made a critical study of the methods in Fitting's own laboratory. He found that the errors of the method could be considerable, as much as  $\pm 32.5$  per cent. He was also unable to establish any definite effect of light on the permeability.

Zycha's work has been criticized by Lepeschkin (1930), who pointed out that Zycha did not use the same slice of epidermis for the determination of the isotonic coefficients as Lepeschkin had done, but used two parallel rows of slices. He considered the method to be insufficiently accurate, since the concentration of the cell sap of epidermal cells is comparatively small and varies too much, whereas the permeability of protoplasm is not great enough. He contended that Zycha was therefore unable to observe the difference between the isotonic coefficients in light and in darkness.

The effect of light on the plasmatic permeability has subsequently been the object of a number of investigations. In some of them, however, this question has only been a detail in a larger problem. Thus, for example, studies of the stomata mechanism have often included an investigation of the association between light and permeability, on the grounds that the guard cells of the stomata are sensitive to light. The physiological processes in such highly specialized cells are, however, complicated and far from fully elucidated. It is therefore practically impossible to determine whether an individual reaction can be considered as a cause or as an effect of permeability or whether it has any association whatsoever with this factor. No account of such investigations will therefore be given in the following.

Ruhland (1912) and Ruhland and Hoffmann (1925) made use of the

plasmolytic method. Ruhland (1912) studied the permeability of leaves of *Beta vulgaris* to glucose and fructose both in sunlight and in darkness. Plasmolysis covered a period of 24 hr. In general, it was not possible to establish any definite and measurable increase in the permeability under the influence of light, but some of the experiments possibly pointed in the same direction as those of Tröndle.

Ruhland and Hoffmann (1925) used *Beggiatoa* as the test object. They were also unable to find any definite effect of light. Meindl (1934), on the other hand, found that the permeability of *Elodea* to urea was up to 50 per cent higher in sunlight than in darkness.

A modification of the plasmolytic method is based on the plasmometric method of Höfler (1918). It consists in the observation of the rate of deplasmolysis during the slow penetration of a substance in the solution.

Hoffmann (1927) used the afore-mentioned method in an investigation of the effect of light on the permeability of the cells of *Spirogyra* to glycerol. He found that it was approximately 20-30 per cent lower in the dark.

Hofmeister (1935) made use of the same method in a study of the intake of glycerol and urea by *Zygnema*. He was unable to establish any effect of light on the intake of these substances.

Wahry (1936) tested the permeation of about twenty organic substances in the submerged and aerial leaves of *Hippuris*, using the plasmometric method. His experiments showed that, as a rule, light hastened the penetration of these substances into the submerged leaves, whereas the effects varied in the case of the aerial leaves. The intake of certain substances was increased in light, in others there was a decrease, and in some cases the intake was unaffected. Thus the reaction differed in the two types of leaves. Järvenkylä (1937) raised the objection to this investigation that the experiments had not been performed under uniform conditions of illumination, that no parallel experiments had been made simultaneously in light and in darkness, and that therefore they could not be considered as comparable.

*Experiments with the Turgor Method.* Important contributions to the study of the effect of light on the permeation both of water and of solutes have been made by L. and M. Brauner. M. Brauner (1932) initially used the same test object as Lepeschkin, i.e., the pulvini of *Phaseolus multiflorus*. She studied their reaction to light in several media, such as air, water, cane-sugar solution, and paraffin. The changes in the turgor of the cells of the pulvini were used as an indicator of the intake of water by the cells. She found that the direction of the photonastic reaction was dependent on the suction force of the medium. Thus unilateral illumination with 1000 m-c caused a positive reaction in air but a negative one in paraffin and in water. Because the turgor of the illuminated side decreased in air but increased in both the liquid media, it was concluded

that the permeability to water is altered by light, the rate of exchange of water being hastened.

Lepeschkin (1934) objected to this conclusion on theoretical grounds. He pointed out that the new state of equilibrium established by the movements of the leaf implies a change in the permeability to the dissolved constituents of the cell and that this cannot be caused only by a change in the permeability to water.

In order to throw light on the nature of the photopermeability reactions involved, Brauner and Brauner (1947) made experiments with the pulvini of *Robinia pseudoacacia*. The pulvini were illuminated under water, and their reactions compared with the effect of the same light stimulus on pulvini in air. Whereas in air white light of all intensities caused positive reactions only, the sign of the response of the submerged pulvini depended on the strength of the stimulus. A low-intensity light produced negative reactions, and a fivefold higher one, positive reactions. It was concluded from the experiments that illumination with moderate quantities of light only increases the permeability to water in the sensitive cells. In air this change was considered to cause an increase in the loss of water by transpiration, and in submerged organs, a transitory increase in the water absorption from the medium. Stronger stimulation, however, led to a partial breakdown of the semipermeability which, both in air and under water, resulted in a loss of suction force and hence of turgescence.

In a new study of *Phaseolus multiflorus* Brauner (1948) used colored light of fixed quality. It was found, among other things, that red and blue light had the same effect as white light, whereas green light was inactive.

*Experiments with the Method of Chemical Analysis.* In a number of investigations the interpretation of the results has been based on an analysis of the solution from which the test object imbibed salts or other substances, or an analysis of the content of such substances in the cells or their sap.

Brauner and Brauner (1936) also made use of this method, their test object being the root parenchyma of *Daucus carota*. In order as far as possible to obtain natural conditions, they used the cell substances of the object, particularly mono- and disaccharides, as the test substance. After illumination an analysis was made of the sugar content of the cells and of the solution.

The ratio between the quantity of sugar given out during a fixed period and the initial sugar content of the cells was taken as a measure of the permeability. The ratio between the amount of sugar given off in darkness and in light was used as a measure of the effect of light. It was found that light decreased the permeation of sugar at all the intensities tested within the range 156-5000 m-c and that the maximal effect

occurred at 625 m-c. The large disaccharide molecules were affected to a greater extent than the monosaccharides.

Approximately at the same time Steward (1932) studied the effect of light on the intake of bromide ions by potato tissue but was unable to demonstrate any influence.

In test objects with large cells it is possible to study the permeability by means of a direct analysis of the cell sap. The salt intake of *Nitella* was investigated in this way by Hoagland and Davis (1923) and by Hoagland *et al.* (1926). They found that illumination enhanced the intake of chlorine, bromine, and nitrate. Thus the sap of plants that had been exposed to light had a higher content of these substances than did the sap of those which had remained in the dark for a similar period, this varying from a few hours to 5 days. According to the afore-mentioned authors, this difference was dependent not only on the permeability of the protoplasm but also on the ability of the cell to utilize the light energy for the work involved in the transport of salts.

Hoagland and Davis called attention to the fact that most of the inorganic elements of the cell sap are present in dissociated form and that the cells are able to cause the movements of ions from a solution of low concentration into one of higher concentration. This condition requires that energy relations be taken into account. In other words, the plant must apparently do work in absorbing ions from dilute solutions. Hoagland and Davis presumed that, in the case of autotrophic plants, light—either directly or indirectly—is necessary for the process of absorption. This is because light furnishes the ultimate source of energy to the plant.

Jacques and Osterhout (1934), working with *Valonia* under different illuminations and in dark, found that, in all cases in which a pH difference was maintained, the rate of intake of potassium was greater at the higher pH. Since the value of the hydroxyl group just outside the protoplasm would increase as a result of photosynthesis, they assumed this process to be the primary cause of the phenomenon.

In a later work Jacques (1939) returned to the same view of the pH effect on rate of intake. He studied the entrance and exit of ammonia in daylight and in darkness, using *V. macrophysa* as the test object. After exposure, analyses for potassium, sodium, and ammonia were carried out on small samples. He stated that accumulation of ammonia takes place more rapidly in light than in darkness and that the accumulation appears to continue until an equilibrium is attained. He found that the equilibrium concentration of ammonia was approximately twice as great in light as in darkness. According to Jacques, both effects may be due to the fact that the external pH—and hence the concentration of undissociated ammonia—is raised by photosynthesis.

The exit of accumulated ammonia from the sap of *Valonia* into normal

sea water was also studied in daylight and darkness. After exit had started, it was found to be greater in light than in darkness.

Ingold (1936), who made similar experiments with *Elodea*, found that light increased the absorption of salts by the shoots immersed in water. He referred to Hoagland's afore-mentioned hypothesis for an explanation of his findings.

If the interpretation of the results obtained by Jacques *et al.* is correct, the effect of light on the intake of substances would, in these instances, be indirect only. The experiments would then rightly belong in the category first mentioned in the present survey (see section on Indirect Influences).

Arisz (1947) studied the intake of salts—particularly of chlorides—by leaves of *Vallisneria*. Because the wounds of the cut leaves influenced the intake, the leaves were pretreated in distilled water for 24 hr. After that time the influence of the wounds had subsided to a great extent. As a result, it was found that the absorption of chlorine from a balanced solution of potassium chloride and calcium sulfate depended on the concentration of chlorine in the external fluid, on the pH of the solution—pH 4.5–9 being detrimental—and on the light. Exposure to light had a remarkable effect on the strength of the absorption. The stronger it was, the stronger the absorption. Light did not influence the intake by photosynthetic processes, since it was also active when carbonic acid was lacking from the medium. Arisz referred to the investigations of Hoagland and coworkers (Hoagland and Davis, 1923; Hoagland *et al.*, 1926), Jacques and Osterhout (1934), and Ingold (1936) and their conclusions regarding the connection between photosynthesis and the influence of light on permeability. Because Arisz's experiments with *Vallisneria* showed that light had the same influence in a medium free from carbonic acid—i.e., without photosynthesis—their conclusions cannot be accepted in the case of this plant.

Phillis and Mason (1937) found that cotton leaves absorbed sugar from a sugar solution and formed starch only in the light and when supplied with oxygen. This process also took place in a medium free from carbonic acid.

Collander (1939) investigated the effect of light on the intake of cations in *Chara ceratophylla* and *Tolypellopsis stelligera*. Parallel tests were made with plants both in diffuse daylight and in darkness in salt solutions of approximately the same concentration as in the natural state. After a few days the cell sap of the internodal cells was tapped off and analyzed. The experiments showed that absorption—at any rate, of lithium, potassium, rubidium, and strontium—was stimulated by light. This influence was considerably stronger in the case of lithium than in that of rubidium. It was also stronger in the case of *Tolypellopsis* than in that of *Chara*. The amount of lithium taken up by *Tolypellopsis* in

diffuse daylight sometimes exceeded that absorbed by similar cells in the dark by as much as six times. No difference could be found with regard to calcium and manganese.

A thorough study was made by Järvenkylä (1937). He used the epidermal cells of the underside of leaves of *Rhoco discolor* and the leaf cells of *Elodea densa* and *Chara ceratophylla*. The two first-mentioned objects were examined plasmolytically, *Chara* being used for analyses of the cell sap. The permeability was tested to a number of substances, such as ethylene glycol, glycerol, trimethyl citrate, urea, methyl urea, monacetine, hexamethylenetetramine, malonamide, potassium nitrate, and lithium chloride.

The influence of light on the permeability varied with the test object, the wave length, and the nature of the substance. A 300-watt lamp at a distance of 41 cm caused an appreciable increase in the rate of permeation of the substances in the case of *Elodea* and *Chara* but had no effect in the case of *Rhoeo*. In the last-mentioned plant a positive effect of light was obtained only in direct sunlight. The effect of light on the permeability increased with decreasing wave length, mainly 450-600  $\mu$ .

The reactions differed for the substances studied. The influence of light was greatest in the case of hexamethylenetetramine, the permeability to which was 80 per cent higher in the light than in the dark, whereas the increase was only 10 per cent in the case of trimethyl citrate. The other substances studied by Järvenkylä had values between these two extremes.

Järvenkylä also made the interesting observation that the effect of light decreases with an increase in the solubility in lipoids and with an increase in the size of the molecules of the permeating substance. He explained this connection by the assumption that light loosens lipoids in the plasmatic membrane.

Osterhout (1947) expressed the view that the increased intake of non-electrolytes in *Chara* noted by Järvenkylä could not be due to a supply of energy, since there was no accumulation of the penetrating substance and it does not seem possible that the pH was involved. He considered that there might be a direct effect on permeability or on the cell wall.

Järvenkylä's experiments also showed that a longer period (3 days) in the dark increased the permeability. It is therefore possible that, in a comparison between the permeability of objects that have stood in the light and the permeability of those which have stood in the dark, the two methods of treatment may give approximately the same results. This may then be interpreted as evidence that light has no influence, although in both cases the permeability has been altered by the light factor. Thus the pretreatment of the object is of decisive importance for the results of the experiments.

In this case the similarity between the dependence of the permeability

and that of the viscosity on light is evident. Also, for the influence of light on the viscosity, the pretreatment of the object is of decisive importance.

*Experiments with Absorption of Dyes.* Measurements or estimations of the ability of the cell to absorb dyes have been used in studies of the association between permeability and the influence of light. The method is simple and convenient but unfortunately involves the risk of confusing the effects of permeability and the ability of the cell to store the dye. The cell contents may show strong stainability on the grounds not only of good permeability but also of the great ability of the cell to bind or precipitate the dye.

Segel (1915) used this method to study the permeability of *Elodea densa* in light and in darkness. He found that light promoted the intake of methylene blue and of neutral red. The experiments covered a period of 3–8 hr. Efimoff and Efimoff (1925), who made similar experiments with a number of dyes, found that only methylene blue was absorbed in light more markedly than in dark. But according to Lepeschkin (1930), he did not consider the chemical changes in aniline dyes to be produced by light. All those dyes in the Efimoffs' experiments which failed to show any effects of light are particularly sensitive to it.

Lepeschkin (1930, 1932a,b), who used the same method, covered part of the leaves of *Elodea* with tin foil so that the same leaf could be exposed simultaneously to light and darkness. In this case as well, the intake of dyes was enhanced by light. Staining after a longer period (100 min) gave the same results in both types of plants, whereas initially stainability was greatest in the plant exposed to light. Lepeschkin therefore considered this as proof that the stronger stainability of the object or part of the object exposed to light was actually due to an increase in the permeability and not to an increase in the storing ability of the cell.

The rays most active in producing the increase in permeability were those with a wave length of 320–420  $m\mu$ ; violet rays were less active, blue and green still less active, and red rays the least active.

Packard (1925) stained paramecia with neutral red and then transferred them to a weak solution of ammonium hydroxide. As the ammonia entered, it changed the red granules to yellow at a rate that was constant under definite conditions. In the ammonia all the cells did not lose their red color simultaneously. If the process was watched under a binocular microscope, each cell could be pipetted off as soon as it was decolorized and the time noted which had elapsed after it had been put into the ammonia. When all the cells had been withdrawn, the average time for decolorizing the total number was calculated. The experiments showed that light was an important factor in producing changes in the permeability of *Paramecium* to ammonium hydroxide. In light the permeability was greater than in darkness, the change also being found

in cells exposed to monochromatic red light. It became greater as the wave lengths shortened and was greatest in the near-ultraviolet range. The duration of light was also an important factor. When paramecia were kept in darkness for 2 hr or more and then exposed to light for periods ranging from 1 to 5 hr, it was found that the velocity of color change increased rapidly after a 1-hr exposure and then more slowly, until after 5 hr there was practically no further increase.

Brooks (1927) studied the absorption of 2,4-dibromophenolindophenol in light of different wave lengths (300–700  $m\mu$ ). *Valonia macrophysa* was used as the test object and placed in glass dishes containing a 0.00035 *M* solution of the dye dissolved in sea water. The dishes were then placed in larger pans, whose tops were screened with glass screens of different colors (red, green, or blue) or uncolored, and the pans placed in diffuse daylight. After the end of 1, 3, 5, and 10 hr, a number of plants were removed and tested by analyzing the cell sap. It was assumed that equilibrium conditions were set up between the external solution, the protoplasm, and the sap at the end of the experiment. The results showed that the amount of dye in the sap increased as the wave length of the incident light decreased toward the ultraviolet end of the spectrum. Thus, the results obtained by Brooks (1927), Packard (1925), Lepeschkin (1930, 1932a,b,c), and Järvenkylä (1937) are in agreement with regard to the association between the influence of light and the wave length.

*Experiments with Absorption of Poisonous Substances.* Kahho (1921) treated sections of red cabbage with solutions of salts with a slightly poisonous effect (sodium iodide, sodium bromide, and sodium thiocyanate) and determined the number of damaged or dead cells with the plasmolytic method. In a comparison between sections treated in the light and those which had remained in the dark, it was found that damage to the cells was more extensive in the former case. This was interpreted by Kahho as an indication that light increased the permeability of the cells to the salts in question.

It is not only the permeability of the protoplasm of plants and lower animals which is influenced by light. Lepeschkin (1932c, 1933) found similar reactions in the erythrocytes of animals and man. Light produced a greater intake of glycerol into the erythrocytes, resulting in a more rapid swelling and hemolysis of the cells than when the erythrocytes were kept in the dark. When exposed to light, human erythrocytes absorbed more glucose and increased more in volume than in the dark. The permeability to sodium chloride was also found to be weaker in the dark.

*Experiments with the Method of Electrical Resistance.* Rapid methods are of great importance for a study of the relation between light and permeability. If, for example, the permeability is affected by the changes in viscosity induced by light or by other processes associated with them,

this can give rise to rapid changes in the permeability which cannot be registered with methods with a slow action.

The method used by Blackman and Paine (1918) to determine the exit of electrolytes from the cells by measuring the electrical conductivity of the surrounding fluid therefore presents considerable advantages over slower methods. However, it permits a determination only of the difference between the intake of electrolytes by the cells and their output. If both processes are equal, no results are obtained with the method. Blackman and Paine used the method to study the photonastic reactions of the pulvini of mimosa, for pulvini immersed in water cause a change in its electrical conductivity.

It was shown that contraction of the pulvinus was associated with an exit of electrolytes from it and that the permeability of the cells was markedly increased by exposure to light, the effect decreasing rapidly with time. A sudden change from light to darkness also increased the permeability. It was therefore not the light in itself, but the changes in light, which induced the changes in permeability.

The same method was used by Dillewijn (1927). He placed severed hypocotyls of *Helianthus* in distilled water and measured the electrical conductivity of the water. The experiments were started 8–10 hr after severing of the hypocotyls in order to avoid the interferences accompanying the intervention. In objects that had stood in the dark and had then been illuminated with  $200 \text{ m-c} \times 400 \text{ sec}$ , the permeability showed a decrease during the first 20 min, with a rapid increase during the subsequent 7 min. This in turn was followed by a decrease and some variations, which gradually ceased. These changes in permeability are reminiscent of the changes in viscosity caused by illumination of objects that have earlier stood in the dark. It would scarcely have been possible to demonstrate their occurrence with the use of slowly working methods such as that of plasmolysis or an analysis of the cell sap.

Lepeschkin (1948) placed leaflets of *Sambucus nigra* and *Parthenocissus quinquefolia* on the surface of dilute solutions of  $\text{CaCl}_2$  (0.0006–0.0009 *M*) or of a mixture of tap water and water distilled in quartz, with the upper side (without stomata) downward. He then determined the electrical resistance of these solutions and its variations. The exit of salts from the leaves was greater by day than by night and was accelerated not only by direct but also by dispersed sunlight.

*Summary of the Experiments.* The investigations described in the foregoing amount to about 40 and were made with at least 7 different methods, the number of test objects being approximately 25. In general, they showed that light increases the passage of substances through the protoplasm and membranes, i.e., that it increases the permeability of the cells. In only a few cases was it impossible to show any such effect. The agreement between the results must be denoted as good, if the con-

siderable variations in the experimental conditions are taken into consideration. Such variations occurred in the following respects, among others:

1. The objects varied not only in species but also with regard to the tissues and organs, age, and degree of development. The importance of these variations has been particularly stressed by Arisz (1947). He therefore warned against drawing conclusions from the data concerning one object as to the behavior of others. It was also evident from Wahry's (1936) experiments that leaves on the same plant may react differently under apparently identical external conditions.

2. The pretreatment of the objects varied, thereby causing a lack of uniformity in the initial conditions. The fact that pretreatment influences the manner of reaction of the test object has been shown by the experiments of Tröndle (1910), Lepeschkin (1930), and Järvenkylä (1937), among others.

3. The quality and intensity of the light and the length of illumination varied in the different experiments. The results are influenced by variations in these factors (cf., for example, Brauner and Brauner, 1936).

4. The permeating substances studied varied. The influence of light on their permeation was therefore subject to variations (cf. Järvenkylä, 1937).

5. The duration of the experiments varied between a few minutes and several days. If, as shown by Dillewijn (1927) and Lepeschkin (1930), the influence of light is increased and decreased in wavelike periods, the choice of the duration of the experiment will be decisive for the final results.

Despite the afore-mentioned variations in the experimental conditions, the results showed good agreement. It may therefore be considered as an established fact that light changes the permeability of the cells, that this change usually consists in an increase in the permeability, and that the effect increases with a decrease in the wave length. With regard to the last-mentioned conclusion, there has been agreement between all the experiments made with a view to analyzing this relation (Packard, 1925; Brooks, 1927; Lepeschkin, 1930, 1932a,b,c; Järvenkylä, 1937).

Thus the experiments of Lepeschkin (1908a,b, 1909) and of Tröndle (1910), which were subjected to many objections and considerable criticism in the years following their publication, have been confirmed after forty years' research.

*Mechanism of Influence of Light on Permeability.* An attempt to analyze the nature of the photic mechanism presupposes, in the first place, a distinction between the direct and indirect influence of light on the intake and output of the substances. The experiments first described here form examples of the indirect influences. They can presumably be explained on the grounds of, among other factors, an increased consump-

tion or production of a substance with resulting changes in concentration. Such processes give no support to the assumption that changes in permeability have occurred. In the great majority of experiments, however, the conditions have been such that the effects of light indicated a direct association between this factor and the permeability of the plasma.

A number of workers have put forward hypotheses or assumptions regarding the manner in which the effect of light occurs. Systematic experiments to determine the nature of the mechanism have, however, been made in only a few instances.

According to Lepeschkin (1930, 1938), the action of light can be explained by the supposition that the change in permeability produced by it is a photochemical process, and that the cells are able to restore some substances important for the selective permeability of protoplasm and destroyed by light. Both processes may proceed in light, but if the cells are transferred to the dark, only a restoration takes place.

As support for his hypothesis, Lepeschkin (1938) referred to the changes undergone by leukocytes on exposure to light. These were manifested, for example, in their reaction to staining of the protoplasm. He also mentioned the hemolysis taking place in red corpuscles in light.

Lepeschkin also considered his theory to be substantiated by other experiments. He found (1940) that light influenced the movements of salts from potato discs immersed in water toward their outer side ("exosmosis"). The net exit or the net accumulation of salts was determined by the method of the electrical conductivity of weak salt solutions in which the objects were immersed. The exosmosis was increased by direct sunlight and by the rays of a quartz-mercury-vapor lamp, i.e., chiefly by ultraviolet rays, whereas electric incandescent light (a 200-watt lamp) did not influence it noticeably. Lepeschkin assumed that light probably destroys the most unstable lipoprotein complexes ("vitoids") participating in the maintenance of the semipermeability of the cell protoplasm and that these destroyed vitoids are replaced in the dark by synthetic processes in the cells. In this connection, Järvenkylä's hypothesis (1937), mentioned earlier, i.e., that light loosens the lipoids in the plasmatic membranes, may be recalled.

Thorough studies of the photic mechanism were made by Brauner and Brauner (1937). They noted that asymmetric illumination gave rise to electrical potentials and changes in the potentials in *Elodea densa*, the illuminated part, or that most illuminated, being positive as compared with the unilluminated or less illuminated part. In order to elucidate the mechanism, they (1938) arranged special experiments of the photoelectric reactions in models of membranes. These led to the conclusion that the mobility of cations and of at least one voluminous anion ( $\text{PO}_4^{3-}$ ) seems to be considerably decreased within the illuminated boundary layers of these systems.

Thus Brauner and Brauner (1940) referred to the main principles of photochemistry. According to these, the primary effect of light on photosensitive systems consists in a separation of electrons from the absorbing surface, which in turn implies a loss of negative charge by the irradiated body. If the system, like the membranes in the aforementioned experiments, is originally negatively charged, then this loss must mean a more or less far-reaching discharge of the pore structure. This in its turn would be liable to affect the passage of water and solutes in two ways, differing in principle:

1. Since a discharge of the micelles building up the boundary layers must lead to a reduction in their mutual electric repulsion, their cohesion forces will prevail and cause a contraction of the illuminated structure and a narrowing of the pores and intermicellar spaces; or this constriction of the intermicellar spaces will follow as a result of a reduced degree of swelling in the membranes caused by the discharge of the micelles and the ensuing loss of hydration.

2. The behavior of water cannot, however, be explained by this conception. For the permeability of water three different possibilities must be taken into account: (a) an increase in the osmotic capacity of the tissue or a decrease in the wall pressure; (b) a change in the friction to which the water threads are subject when moving through the pores of the membrane. In an illuminated system the friction could be expected to increase as a result of the reasons given under paragraph 1, but this effect would possibly be compensated by the decrease in the electrostatic braking forces of the pore walls after their partial discharge; (c) the participation of an electroosmotic process in the meaning of Loeb (1920a,b, 1921). Such an additional force would interfere with the normal osmotic suction force of the tissue and, according to its direction, accelerate or retard the velocity of the water flow. Since, according to the aforementioned experiments on *Elodea* and model systems, preexisting membrane potentials are affected by illumination, if the diaphragm is light-sensitive, light may influence the water intake indirectly by modifying the electroosmotic component of the tissue.

In order to permit a decision between the different possibilities discussed in the foregoing, Brauner and Brauner (1940) made special experiments on root tissue of *Daucus carota*. Using a gravimetric method, they investigated the water intake and output in light and darkness, in distilled water, in hypertonic and hypotonic solutions, and during different time intervals.

In distilled water the photoreaction appeared as an increase in the rate of water intake, its extent varying with the exposure time. The magnitude of the reaction reached its maximum after about 30 min (light intensity  $36 \times 10^{-3}$  cal/cm<sup>2</sup>/sec; initial temperature 25°C).

In hypertonic sugar solutions (0.5 and 0.7 *M* glucose) the water loss of

the tissue was unaffected by illumination. It was concluded that strong osmotic agents destroy the sensitivity of the system to light.

Hypotonic electrolyte solutions affected the photoreaction of the water intake in a characteristic way. It could best be explained by the assumption that the electrical diffusion potential between tissue and medium plays a decisive role in the process. Comparing these results and their previous experience with the photoelectric effect in organic membranes, the authors found it most probable that the light-sensitive factor of the water intake is its electroosmotic component.

Thus it was necessary to attribute the surplus water intake observed in illuminated samples to the appearance of an additional motive force, rather than to an increase in the water permeability itself. At any rate, the part played by the latter factor remained unsubstantiated.

It has been shown that an antagonism is to be expected between the electrostatic field effect of the membrane charge (primary photoeffect) and the mechanical filter effect of the pores, depending on the degree of hydration of the membrane substance (secondary photoeffect). A loss in charge would facilitate the transport of water by reducing the electric braking force of the membrane, but at the same time it would have a delaying effect by a constriction of the pore diameter, owing to a decrease in the swelling power of the micellar structure.

If this view of the mechanism is correct, the velocity of the water flow should be controlled mainly by the electrostatic factor when the intermicellar spaces are wide, but by the mechanical effect when they are narrow.

Brauner and Brauner (1943) also made an attempt to investigate this important question. They kept the ion concentration uniform throughout the system by using a homogeneous membrane (cellophane type) instead of a living cellular structure. They thus avoided the development of an electroosmotic component. The electric charge of the diaphragm was influenced by varying the pH of the medium and not by irradiation, thus making it possible to investigate a much wider range of charge conditions and, moreover, to test membranes insensitive to light as well. Instead of osmosis, a fixed hydraulic pressure was used to force the liquids through the membrane.

In a second part of the investigation a study was also made of the relation between the water permeability of living plant cells (storage tissue of potato and red beet root and epidermal cells of *Allium cepa*) and the pH of the ambient medium.

It was concluded from these experiments that the water permeability of plant cells in a state of normal hydration is regulated mainly by the electrostatic valve effect of the micellar charge, and that the mechanical filter control becomes operative only after artificial densification of the protoplasm, e.g., after previous plasmolysis.

According to Brauner and Brauner, the effect of light is due, among other factors, to the changes caused by it in certain details of the plasmatic structure, thus leading to changes in the cellular permeability. The importance of the plasmatic structure for permeability has been particularly stressed by Seifriz (1945). He stated:

The selective permeability of cells is generally handled as a subject apart from structure, but several of the classical theories of cellular permeability involve mechanisms, and mechanisms involve structure. Thus the sieve hypothesis of selective permeability is a purely mechanical interpretation; indeed, chemical hypotheses such as those involving the solution of fats are, in the last analysis, structural interpretations.

Thus the analysis of the influence of light on the permeability of the protoplasm appears to point to the plasmatic structure and to properties of the protoplasm associated with it. According to the analysis of the influence of light on the viscosity of the protoplasm and to the account already given of this question, there is also in this case reason to seek the explanation in the plasmatic structure.

*Association between Effects of Light on Permeability and Viscosity of Protoplasm.* As mentioned earlier, the influence of light on the permeability resembles, in some cases, the alterations in viscosity caused by light. Lepeschkin (1930, p. 964), working with *Elodea canadensis* and *E. densa*, found that a periodic variation in permeability occurred in plants that had been kept in darkness, and that these variations continued for 3 days, after which the value remained almost constant. With increasing light the permeability also increased, but not proportionately. At a light intensity of about 10 per cent of sunlight, the permeability reached its maximum, a subsequent increase in the intensity having no effect. Similar changes in the permeability following the action of light were also found by Dillewijn (1927).

Indeed, these observations on the permeability correspond to the changes in viscosity caused by light, as described in the foregoing. This seems to suggest a correspondence between viscosity and permeability, and may be interpreted as supporting the hypothesis of a causal connection between the alterations in permeability and viscosity or of both phenomena as consequences of a third alternating factor.

It is necessary for studies of such a connection between permeability and viscosity to use methods that permit determinations during short periods of time. Most of the methods used hitherto require considerable time and are therefore unable to allow the details of the influence of light to be observed.

*Physiological Consequences of Effect of Light on Permeability.* It can be expected that light, by influencing the permeability, will cause impor-

tant physiological processes in plants under natural conditions. An example of such processes has been reported by Heimann (1950), who stated that the guttation of *Kalanchoe blossfeldiana* changed in the same rhythm as natural daylight and that it adapted itself if the intervals between light and darkness were altered. He assumed that the primary cause was to be found in the action of light on the permeability of the protoplasm.

#### INFLUENCE OF VISIBLE LIGHT ON PROTOPLASMIC STREAMING

Pringsheim (1882) studied the protoplasmic streaming of *Nitella*, *Tradescantia*, and *Spirogyra* and found that the streaming could be started by light. Moore (1888), who used *Vallisneria spiralis* as the test object, and Schröter (1905) and Andrews (1912), who used species of *Mucor*, came to the same conclusion. Nothmann-Zuckermandl (1915) subsequently made a more detailed analysis of the question. His tests were made on leaves of *Elodea* and *Vallisneria*, the stamen hairs of *Tradescantia*, and the root hairs of *Hydrocharis*. Gaslight or an arc lamp was used as the source of light, the heat rays being removed with various forms of filters. His experiments showed the existence of certain quantitative associations between the reaction and the strength or quantity of light. The stronger the light, the more rapidly did the protoplasmic streaming start, and the longer were the aftereffects of illumination. Illumination with an arc lamp for 2–5 min started a streaming that continued for 6–24 hr. The accuracy of these conclusions was later confirmed by Fitting (1925). The reaction was also found to be dependent on the wave length of the light. Nothmann-Zuckermandl compared different wave-length ranges after the light had been screened off to the same strength (measured with a thermoelement). He found that red and infrared light had the greatest effect, that the other kinds of rays were less active, and that their effect diminished with the wave length.

Beikirch (1925) and Schweickerdt (1928) also studied the influence of various kinds of light. They were, however, only able to confirm Nothmann-Zuckermandl's results with regard to red light, which they also found to be most active. On the other hand, they found infrared light to be inactive, and Schweickerdt's experiments with *V. spiralis* revealed no parallelism between the influence of the kind of light and the wave length. It was found that blue light and then green had, after red light, the strongest effect.

Protoplasmic streaming ceases in the dark and starts again on illumination (Schröter, 1905; Nothmann-Zuckermandl, 1915). Schweickerdt (1928) showed that the streaming also ceases in red and in green light when illumination has continued for some time and that its rate decreases with continuous weak illumination with white and blue light. The

streaming did not, however, cease altogether in the two last-mentioned forms of light.

As a result of the aftereffects of light, protoplasmic streaming continues during the night in the summer, as has been shown by Fitting (1925). During the winter, on the contrary, it stops during the night. The aftereffect of light on the protoplasmic streaming thus resembles its aftereffects on the viscosity. The processes that are started by the daylight continue during the night and, to all intents and purposes, do not cease—at any rate, not during the lightest part of the year.

After protoplasmic streaming has decreased or ceased as a result of continuous illumination, it can be stimulated or restarted by means of an increase in the strength of the light. Moore (1888), Schröter (1905), and Andrews (1912) were able to observe an increase in the rate of streaming with an increase in the strength of illumination, as long as the latter was kept within moderate limits. Greater intensities of light caused cessation of the streaming, as had already been noted by Hofmeister (1867), Pringsheim (1882), and other workers. As a rule, however, this effect of light appeared to be only a secondary effect and a result of the destructive processes caused by illumination.

Mast (1932) studied the effect of partial illumination of *Amoeba*. He found that the different parts of the cell varied in their reaction to an increase in the intensity of illumination. In some cases the streaming of the protoplasm ceased; in others it changed in direction or increased in rate. He also concluded that light causes gelation of the plasmasol adjoining the plasmagel of the amoeba, making it thicker and increasing the elastic strength of the portion illuminated.

Bottelier (1933, 1934) made an interesting observation, namely, that illumination for a short period (3–4 min) can give rise to both an acceleration and a retardation of the protoplasmic streaming. He studied this phenomenon on the epidermal cells of the *Avena* coleoptile and found that the nature of the reaction was dependent on the quantity of light applied. A very slight amount of light (2–11 ergs/cm<sup>2</sup>) caused a retardation, lasting about 4 min, whereas a larger amount (800 ergs/cm<sup>2</sup>) accelerated the streaming. The intensity of the reaction followed the law of products. Bottelier also noted that blue light had the greatest effect, followed by violet, ultraviolet, and green, in that order, and that yellow and red light had no demonstrable effect. His comparisons were based on experiments with the same quantity of energy (in ergs per square centimeter per second).

In connection with his other studies on protoplasmic streaming and its conditions, Fitting (1925) also investigated the effect of light, which he named "photodinesis." He expressed the opinion that protoplasmic streaming is not induced by light alone but by a transition from weak to stronger illumination. Ewart (1903) had earlier noted that a change

between light and darkness accelerated streaming. Using *Vallisneria* as the test object, Fitting demonstrated that the acceleration in protoplasmic streaming was caused only by a change from weak to stronger light and not by a change from strong to weak illumination. He also found that the change in illumination must take place relatively rapidly in order for such an effect to take place, since no change could be noted in protoplasmic streaming if the increase took place slowly. Because only the illuminated parts reacted, he concluded that no impulse was conducted in this process.

Since protoplasmic streaming thus reacts only to a relatively rapid change in illumination, Seifriz (1943) expressed the view that the effect is due more to a shock effect than to the actual influence of light. He stated that any shock, whether due to light, heat, electricity, or mechanical factors, would presumably accomplish the same result. He was unable to show any stimulating effect of light on the streaming in Myxomycetes. He therefore found it possible that tissues, which like the Myxomycetes are commonly exposed to light, are not activated by it and that tissues not naturally exposed to light, such as the inner cells of stems, are more sensitive and respond by an increase in streaming.

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**Electrical Phenomena in Vision<sup>1</sup>**

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*Introduction. The resting potential. The electroretinogram: Evidence from primitive eyes—The vertebrate electroretinogram—The human electroretinogram—Conclusions with regard to the retinal action potential. Optic nerve impulses: Visual responses of primitive eyes—Responses of vertebrate eyes—Conclusions with regard to the responses of optic nerve fibers—Responses of the optic tract, geniculate body, and cortex—The sequence of events from retina to cortex. Electrical stimulation of the visual system: Visual effects of electrical stimulation—Physiological effects—Polarization of the eyeball—Polarization of individual retinal units—Effects of light on electrical excitability. Summary and conclusions. References.*

**INTRODUCTION**

The sense of sight is generally given credit for being our most important source of information about the outside world. It is not surprising, then, to find that vision, more than any other sense, has been studied intensively by physiologists, psychologists, chemists, physicists, ophthalmologists, and many others. Textbooks on vision (e.g., those of Bartley, 1941; Davson, 1949; Hartridge, 1950; Ogle, 1950; Wright, 1947) reveal that there is now a large mass of factual material on the subject, but it is at once apparent that some of the most elementary facts are still unknown. We seem to be in the anomalous position of having a more extensive coverage of complex phenomena such as form and distance discrimination, contrast effects, apparent motion, and visual illusions than of the more basic processes by which sensory cells respond to light and generate impulses in the conducting mechanisms of the nervous system. We are not sufficiently acquainted with the properties of individual retinal receptors to state the processes underlying visual acuity, color vision, or brightness discrimination.

The author of this chapter is strongly of the opinion that new progress in this field must largely depend upon an analytical approach in which the separate events are studied in receptor cells, retinal neurons, optic nerve fibers, and projection and association areas in the higher centers

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of the nervous system. Visual photochemistry provides the starting point for this type of analysis. The great tool for attacking the rest of the problem is that of recording, by electronic devices, the electrical signs of each part of the visual system. This type of research could scarcely have been undertaken until the late 1920's, when the necessary equipment first became readily available. Since that time a relatively small number of laboratories have taken up the slow and technically difficult task of placing electrodes (see Fig. 13-1) at various locations

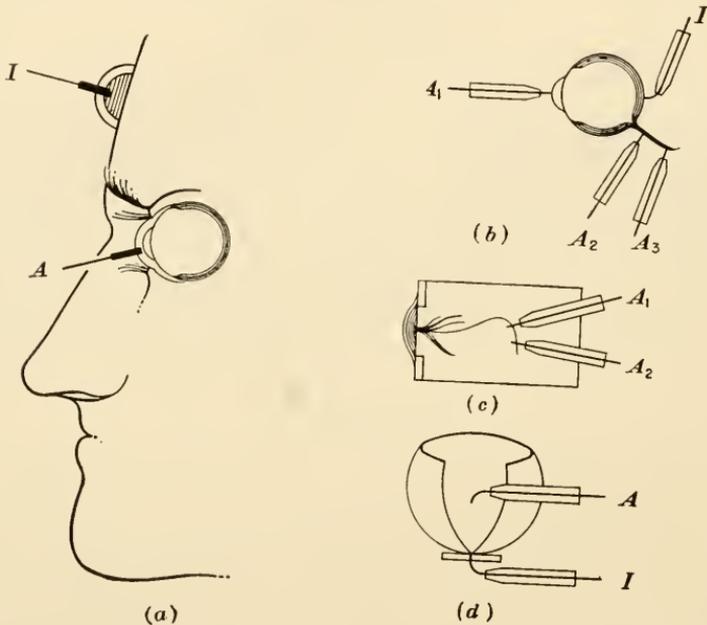


Fig. 13-1. Placement of electrodes for recording electrical responses of the eye. In each drawing *A* is an active electrode, and *I* is an indifferent electrode. (a) Electrodes in position for recording the human action potential by the use of a contact lens. (b) Connections with an excised eye for measuring the resting or action potential. (c) Top view of arrangement for recording impulses from a single fiber dissected out from the optic nerve of *Limulus*. (d) Side view of arrangement for recording impulses from a fiber dissected out from a vertebrate retina.

within the visual system. The resulting data, summarized in this chapter, represent pioneer research in this field. There is reason to believe that this somewhat bewildering array of findings may soon yield the basis for more realistic interpretations of visual phenomena about which there has been seemingly endless speculation.

This chapter will survey the field of electrical phenomena, including the resting potential of the eye; the retinal action potential, or electroretinogram; the responses of optic nerve fibers; records from the optic thalamus and cortex; and effects produced by electrical stimulation of the visual system. It is not possible within the space allotted to give a

historical review of the research in this field. Fortunately Granit's book (1947) and reviews (1950a,b) are available for a detailed summary of his voluminous work and the related work of other investigators. The book by Bartley (1941), the thorough review of the early literature by Kohlrausch (1931), and the later review by Graham (1934) may also be consulted for more comprehensive treatments of these topics.

### THE RESTING POTENTIAL

Every eye appears to exhibit a relatively large (several millivolts) and constant "resting potential." In the case of vertebrates the cornea is normally found to be positive with respect to the fundus, but for invertebrates the polarity is reversed. Vertebrates have "inverted" retinas in

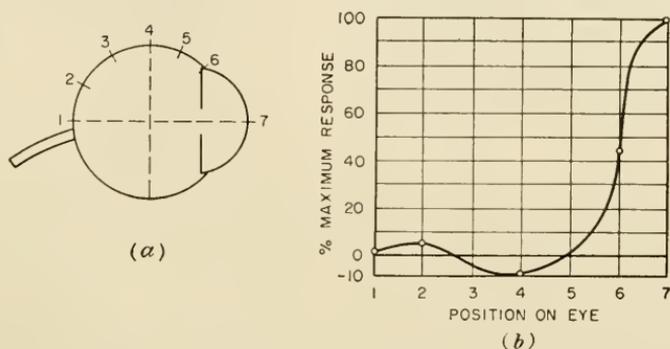


FIG. 13-2. Distribution of the resting potential around the eyeball. (a) The indifferent electrode is at position 1 at the fundus of the eye. (b) The various magnitudes of constant potential recorded between this electrode and an active one at each of the designated points are plotted in the graph. (Redrawn from Kohlrausch, 1931.)

the sense that the rods and cones are pointed away from the light, whereas invertebrate receptor cells point toward the light. Hence we may state the general finding that the constant polarity of any eye is such that the terminal portions of the receptor cells are usually found to be negative in relation to the basal portions.

Many observations support the view that the resting potential of the eyeball originates primarily in the retina. Whether the sensory cells are responsible for it is not known, nor can we be sure that some small part of the resting effect is not extraretinal in origin. Some of the relevant observations are the following: Early experimenters [e.g., Holmgren, Dewar, and McKendrick (see Granit, 1947)] claimed to find a resting potential in isolated retinas. Others [especially de Haas, Westerlund (see Kohlrausch, 1931)] studied the distribution of the resting potential around the eyeball (see Fig. 13-2). They found maximal potential differences between leads on the cornea and fundus. Relatively large differences were found between adjacent regions in the anterior portion of

the eye, whereas the posterior portion of the eye exhibited very slight differences. These findings are consistent with volume conductor effects based on high electrical resistance at the anterior pole of the eyeball. They certainly bear repeating, however, now that electronic recording enables us to distinguish clearly between current and potential differences.

There appears to be some parallelism between the resting potential of the eye and the action potential in response to a flash of light. The polarity of the main (*b*-wave) aspect of the electroretinogram is similar to that of the resting potential in both vertebrate and invertebrate eyes. Thus the *b*-wave appears as an increase in the potential already present, though we must not conclude from this that the two arise within the same retinal cells. More direct evidence is found in Therman's finding (1938) that glucose augments both the action and resting potential, whereas potassium has opposite effects. It is notable, however, that the resting-potential polarity of an excised eye has commonly been observed to deteriorate and indeed become reversed, whereas the action-potential polarity maintains its usual direction. Wulff (1948) has reported evidence that both the resting and action potential of the frog are subject to a rapid decline after the eye has been excised. The ratio of resting potential to action potential is not constant, however, except under conditions where the eye remains fully dark-adapted and is stimulated by relatively weak flashes of light.

Regardless of its origin, the resting potential has been the basis for a convenient method of recording eye movements. Miles (1939a,b) and Carmichael and Dearborn (1947) have studied in detail the changes in polarity between electrodes placed on the skin at either side of the human eye during reading or other activity involving deliberate rotation of the eyes. Although it may be that some external effects are involved here, notably responses of the large external muscles that serve to rotate the eye, a major portion of the effect seems to be attributable to the fact that the positive or corneal pole of the eyeball moves toward one electrode and away from the other for any given rotation of the eye.<sup>2</sup>

### THE ELECTRORETINOGRAM

Relatively slow action potentials are developed by the retina as a whole in an eye that is stimulated by light. Records of this phenomenon may be obtained by placing electrodes directly across the retina or by placing one on the anterior pole of the eyeball and the other at the fundus.

<sup>2</sup> Incidentally in our laboratory we have noted a marked diminution in potentials arising from eye movements in a patient with retinitis pigmentosa. This patient, like others similarly afflicted, also showed an almost complete loss of the action potential. Our observation is therefore consistent with the notion of a common retinal origin for both resting and action potential.

The action potential recorded from these electrodes by oscillographic means, the electroretinogram, is typically a rather complex one whose characteristics depend on the species of animal, the condition of the eye, the electrode placement, the technique used in recording, and the nature of the stimulating light. Such a record from a human eye is shown in Fig. 13-3. There is evidence that the various positive and negative waves of the electroretinogram originate within different retinal structures, but detailed information on this point is lacking. It is clearly not true that the principal component of the electroretinogram is a mere photoelectric phenomenon, nor is it simply a summation of action potentials within optic nerve fibers.

#### EVIDENCE FROM PRIMITIVE EYES

It is not easy in vertebrate eyes to locate the original site of the action potential. In more primitive eyes, however, it appears probable that a large monophasic response originates within the sensory cells of the retina. Hartline (1928) found such a response in the compound eye of *Limulus*, the horseshoe crab, whose ommatidia were supposed to contain only first-order

(sensory) neurones. In *Dytiscus*, the water beetle, Adrian (1937) observed a similar response, though it was complicated by the activity of the ganglia. Bernhard (1942) was able to separate the retina from the other structures in this preparation. The isolated retinal response, presumably from primary sense cells, was always smooth and monophasic. Therman (1940) obtained similar results with *Loligo*, a squid, and observed in addition that there were two separate components in the action potential from this eye. The two components, of opposite sign, are distinct in their responsiveness to chemical stimulation, light adaptation, and other effects. The presence of two distinct retinal systems in another mollusk, *Pecten*, has been demonstrated by Hartline (1938b; see later). It may be that in such an eye each retinal system has its own action potential as well as its own characteristic manner of responding to the onset or extinction of the stimulating light.

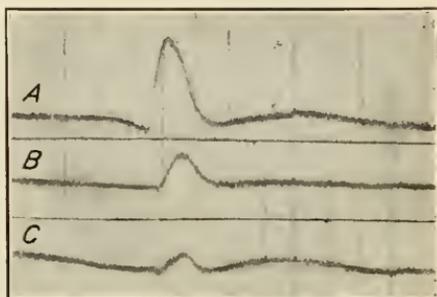


FIG. 13-3. Records of the human electroretinogram. Responses to high, medium, and low intensities of light are shown in A, B, and C, respectively. Time is shown by the faint vertical lines (0.01- and 0.1-sec units). The moment of stimulation is indicated by the gap in the horizontal line at the bottom (flash duration 0.04 sec). In A the first portion of the response is the negative a-wave of very short latency; the principal positive deflection is the b-wave; and the small, second positive deflection is the c-wave. (Unpublished records of R. M. Boynton.)

There is room for some doubt that primary sense cells alone are present in each of the above preparations. Nevertheless, together with work to be described later on single fibers in the optic nerve of *Limulus* (Hartline and Graham, 1932) and on nerve impulses in *Dytiscus* (Bernhard, 1942),

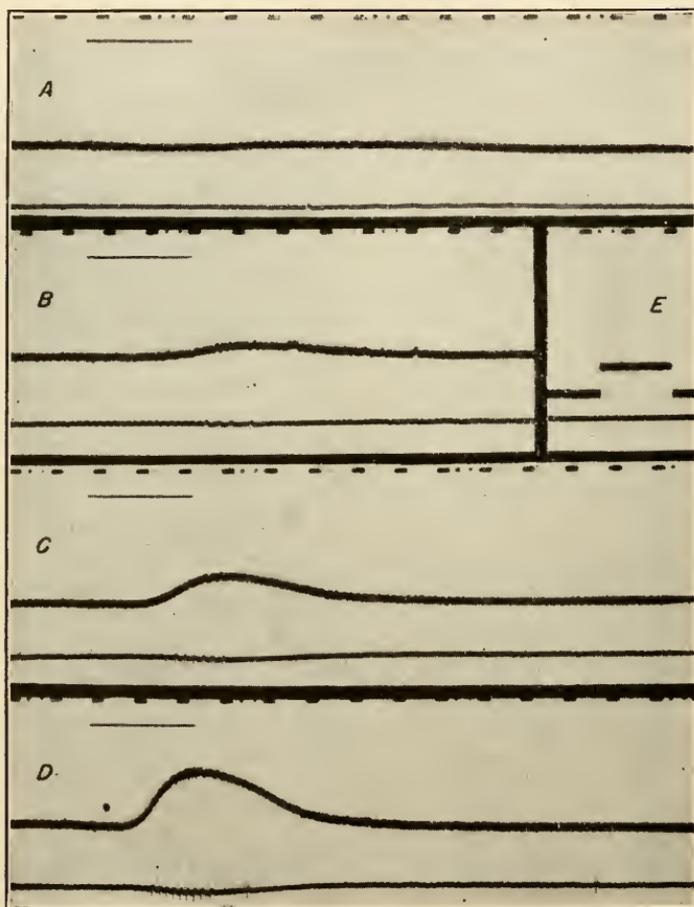


FIG. 13-4. Simultaneous records of action potential and single optic nerve fiber in *Limulus*. In each record the dashed line at the top marks time in tenths of a second; the short horizontal line shows the duration of the stimulating light; the heavy line shows the action potential recorded by electrodes on the front and back of the eye; and the lighter line shows the responses of a single optic nerve fiber. Records A to D denote responses to stimuli of increasing intensity. Record E shows a calibration consisting of a 100- $\mu$ v square wave.

these studies strongly support the following conclusions: (1) The action potential is developed by sensory cells, at least in invertebrate eyes. (2) This action potential is generally of the same polarity as the constant potential; i.e., the terminal portions of the receptor cells become negative to the basal portions; (3) Some aspect of the action potential

begins slightly before the appearance of impulses in the nerve fibers served by the sensory cells. (4) The number and frequency of the nerve impulses are usually related to the magnitude and rate of rise of the action potential. (5) The action potential thus appears to be the sign of a process that has something to do with initiating impulses in the nerve fibers attached to the sensory cells. Figure 13-4 shows the electroretinogram together with a record from a single nerve fiber in the eye of *Limulus*.

#### THE VERTEBRATE ELECTRORETINOGRAM

*Retinal Convergence.* The vertebrate electroretinogram is characteristically less simple than that of invertebrate animals. This seems

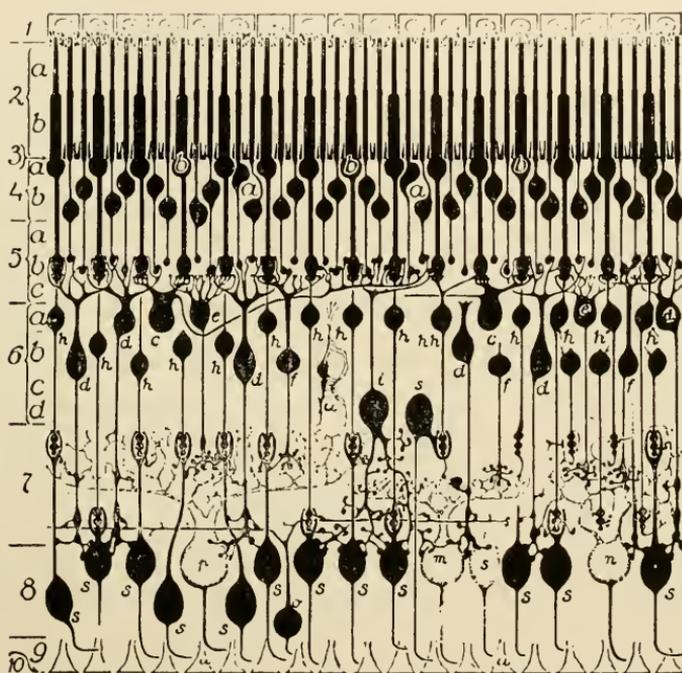


FIG. 13-5. Diagram showing the rod and cone receptor cells (layers 2-5), the bipolar cells and others (layer 6), and the ganglion cells (layers 7 and 8) of the vertebrate retina. (From Polyak, 1941.)

natural enough in view of the fact that the vertebrate retina is comparable to brain tissue in its nervous complexity. Polyak (1941) and others have described mechanisms that provide inhibition and facilitation from one point to another on the retina. Figure 13-5 shows that the optic nerve consists typically of third-order neurones whose cell bodies are in the ganglion layer on the anterior surface of the retina. The second-order neurones are bipolars, running between the primary sense cells (rods or cones) and the ganglion cells. In summary, a single optic nerve

fiber may serve a number of bipolars, each bipolar may in turn be connected with several rod or cone receptor cells, and horizontal interconnecting fibers may carry inhibition or facilitation from one retinal location to another.

*Components of the Electroretinogram.* Many attempts have been made (see Kohlrausch, 1931) to interpret the typical vertebrate electroretinogram on the basis of hypothetical component processes. The most comprehensive of such attempts is that of Granit (1933, 1947). In Granit's analysis (see Fig. 13-6) there are three fundamental processes—PI, PII, and PIII—some of whose properties may be adduced from

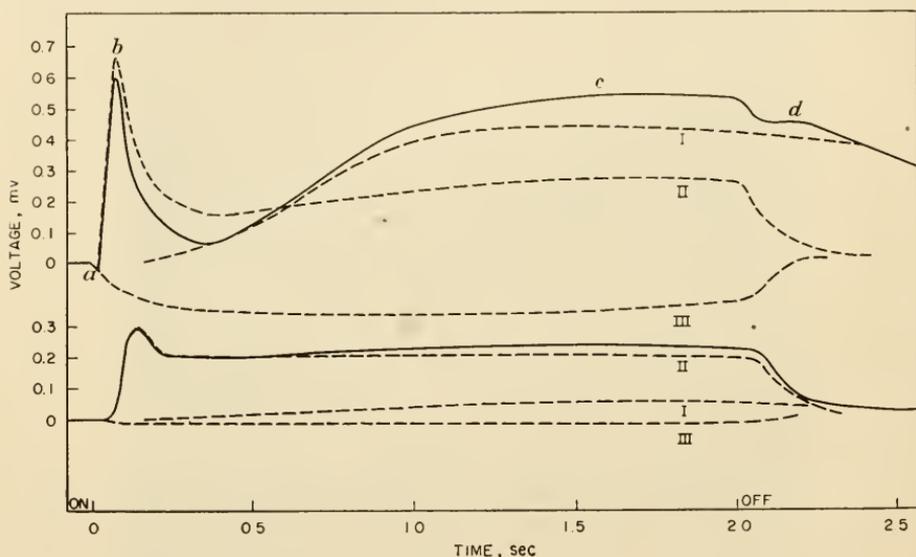


FIG. 13-6. Analysis of the vertebrate electroretinogram according to Granit (1947). Upper heavy line is the electroretinogram in response to a bright light; lower heavy line is that for a weaker light. PI is primarily responsible for the *c*-wave, PII for the *b*-wave, and PIII for the *a*-wave and the *d*-wave (off-response).

Table 13-1. This table is intended, in the interests of brevity, to summarize the most important features of Granit's discussion (1933; 1947, Chaps. 3 and 4).

The assignment of PIII to the sensory cells agrees with the fact that the *a*-wave is often of extremely short latency. Also to be noted is the observation by Piéron and Segal (1939) that this wave is not affected by changes in temperature—a fact suggesting a photochemical origin. Origination in the receptor cells might suggest that PIII represents the event leading to the discharge of nerve impulses in the vertebrate retina. There are two main objections to this suggestion. First, the polarity is wrong, since the invertebrate action potential is a simple monophasic wave showing negativity at the terminal portion of the receptors. Sec-

only, PII (not PIII) appears to be influenced in the same manner by anoxia and drugs as are the nerve impulses of vertebrates.

Although PII in the vertebrate eye has the polarity and other characteristics of the monophasic invertebrate electroretinogram, Granit has cited evidence that it does not originate in either sensory or ganglion cells. Granit and Helme (1939) found that the vertebrate electroretinogram remained normal, while antidromic volleys were aroused electrically in the optic nerve, i.e., in the axons of the ganglion cells. This

TABLE 13-1. PRINCIPAL CHARACTERISTICS OF THE RETINAL ACTION POTENTIAL AS RELATED BY GRANIT TO THREE UNDERLYING PROCESSES

Property	Process		
	PI	PII	PIII
Latent time.....	Long	Medium	Short
Polarity.....	Positive	Positive	Negative
Electroretinogram wave accounted for.....	<i>c</i> -wave	<i>b</i> -wave	<i>a</i> - and <i>d</i> -waves
Effect on nerve impulses..	"Sensitizes" PII	Excitatory	Inhibitory
Result of light adaptation	Not much change	Greatly reduced	Usually abolished
Probable site of origin....	?	Bipolar cells?	Rod and cone cells
Effect of asphyxia.....	Moderately susceptible	Very susceptible	Highly resistant
Effect of ether.....	Abolished first (reversible)	Abolished second (reversible)	Abolished last (irreversible)
Intensity of light to stimulate.....	High	Low	High
Effect of alcohol.....	?	Enhances	Diminishes
Effect of adrenalin.....	Enhances and prolongs	Diminishes and prolongs	?
Effect of KCl.....	None	Abolishes	Enhances, then inhibits

procedure might be expected to depress the action potential if it did in fact originate in these cells. The evidence against rods and cones as originators of the vertebrate action potential lies chiefly in the fact that the principal electroretinogram component (the *b*-wave) has the characteristics of synaptically mediated activity. The *b*-wave, accounted for chiefly by PII, is easily depressed by potassium chloride and by anoxia, but it is augmented by strychnine. Hence the suggestion by Granit that the bipolar cells are a likely source of this principal wave in the vertebrate electroretinogram.

A further extension of this analysis involves Granit's distinction (1935) between excitatory and inhibitory (E- and I-) retinas. E-retinas are considered to be those of mammals, whereas I-retinas are found in all other vertebrate eyes. The I-retina responds strongly to the cessation of light

and hence is particularly capable of responding to a flickering stimulus. It shows a renewed development of PII as the light goes off, as shown by a large *d*-wave in the electroretinogram. In some eyes (for example, in the frog and owl) the retina is of the E-type in the dark-adapted condition but changes to the I-type with light adaptation (Granit, 1947). Marked differences in response characteristics as described by Granit are certainly present, but no such clear dichotomy as the terms "E-retina" and "I-retina" imply has been demonstrated. Ultimately the bases for such distinctions may well be found in the functional or anatomical predominance of rod or cone receptors and in the distribution of retinal elements responding in various ways to the onset and cessation of light.

An interesting hypothesis supported by Granit (1938, 1947) is to the effect that rods and cones are rivals competing for possession of the "final common pathways" in the form of optic nerve fibers. Therman (1939) has appealed to this hypothesis to account for his observation that a given red light may actually arouse a larger action potential in a light-adapted frog than in a dark-adapted one. Red light is relatively (though not absolutely) more effective for cones than for rods in the human eye (see Wald, 1945). If this is also the case in the frog, Therman's finding may lend some support to the rivalry hypothesis.

Of interest here are observations by Granit *et al.* (1939) on the cat. They found that the *b*-wave was dependent upon the presence of a relatively high concentration of visual purple. Even a moderate reduction in this concentration (as by relatively short exposures to light) results in a marked reduction in the *b*-wave. In line with these observations, Riggs and Johnson (1949) have shown that in the human eye very moderate levels (less than 1 ft-lambert) of light adaptation are sufficient to abolish the principal component of the action potential except for stimuli of extremely high intensity. A tenfold increase in level of adapting light is sufficient to cause an increase of nearly a hundredfold in the intensity of a test flash necessary to arouse a given magnitude of action potential.

The reduction in rod-initiated responses by light adaptation is well established. There is no direct evidence for Granit's further hypothesis that cones assert themselves at high intensity levels by taking over the pathways used by rods at low intensities.

It is not possible in this short chapter to present a detailed account of the many experiments on the action potential in various animals. Since this is the only type of electrical recording which has been applied to the human eye, we shall devote particular attention to some of the facts with which it has provided us. Hartline (1925) obtained conclusive evidence that the vertebrate electroretinogram is of the same form whether it is recorded from the excised eyeball or from corneal and neutral leads to the intact animal. He also obtained records of the human electroretinogram and showed that it was grossly similar to that of other mammals.

## THE HUMAN ELECTRORETINOGRAM

A convenient method of recording the human retinal or action potential is by the use of an electrode embedded in a plastic contact lens (Riggs, 1941; Karpe, 1945; Autrum, 1950). This type of electrode may be used to provide a stable and comfortable attachment to the human eye for a period of several hours (see Fig. 13-1).

*Effects of Dark Adaptation.* Wald (1945) has described the remarkable series of events by which the retinal receptors are able to restore the photosensitive substance that is broken down by light. The course of this restorative process may be followed by measuring the height of the human action potential in response to a test flash delivered to the eye at successive intervals. Karpe and Tansley (1948) measured the increase in this height as a function of time in the dark and proceeded to compare their results with the decrease in psychophysical threshold which occurred under the same conditions. They concluded that the two procedures yielded nearly parallel results. They recognized, however, that this parallelism was somewhat arbitrary, since it involved a comparison between a set of response magnitudes (electroretinogram) with a set of stimulus magnitudes (psychophysical thresholds) over a somewhat restricted range of times in the dark.

Johnson (1949) made a thorough study of the effects of dark adaptation on the human electroretinogram. He used red, yellow, blue, and white test stimuli over a wide range of intensities and determined the intensity necessary to produce a given response magnitude at each time in the dark. He found that the *b*-wave of the electroretinogram in man, as in the rat (Charpentier, 1936) and the frog (Riggs, 1937), changes importantly in wave form as well as in magnitude as dark adaptation proceeds. This fact makes it inadvisable to use *b*-wave height alone as an index of state of adaptation (Johnson and Riggs, 1951). Furthermore the *b*-wave steadily increases during the later stages of dark adaptation, by which time the psychophysical threshold has nearly ceased to decline.

Psychophysical data (Hecht, 1934) on dark adaptation show a clear difference between an early stage, for which the cone receptors are believed responsible, and a later stage representing the rod receptors. When red light is used for the test flash in these psychophysical experiments, only the cone portion of the dark adaptation is revealed, since the rods are never much more sensitive than the cones for red stimulation. In the case of the *b*-wave of the electroretinogram, however, the same rate of dark adaptation is shown by red test flashes as by other colors of test flash, though it is necessary to use very high intensities of red to achieve the necessary response (Johnson, 1949). The interpretation of these facts is simple, namely, that the *b*-wave of the human electroretinogram is almost exclusively a product of rod-receptor activity. The

electroretinogram data on dark adaptation are therefore thought to reflect primarily the restorative process in retinal rod receptors, uncomplicated by cone-receptor activity or the activity of higher centers in the visual system.

*Effects of Colored Light.* Color vision is obviously dependent on the ability of the eye to respond differentially to stimulation by lights of various wave lengths. Several investigators have therefore attempted to obtain specific electrical responses of the human eye to colored lights. Motokawa and Mita (1942), using electrodes located on the skin adjacent to the eyeball, were able to observe an "*x*-wave," consisting of a rapid spike in response to red, which preceded the regular *b*-wave of the electroretinogram. It is probable that the conditions of recording were such that other specific effects were not observed.

Adrian (1945, 1946), using a moist thread electrode on the eye and a capacitance-coupled amplifier, was able to obtain a more comprehensive set of records showing responses to colored lights. He found that deep red light produced only a small but rapidly developing action potential in the eye of man or monkey. Orange-red light yielded a double wave consisting of this rapid component plus the regular *b*-wave. Lights of shorter wave lengths produced more typical *b*-waves with little evidence of the rapid component. Adrian concluded that the rapid response was that of a photopic or high-level system, whereas the regular *b*-wave arose from the scotopic system on which we depend for seeing at night. He was able to modify the two differentially by using appropriate conditions of adaptation, flicker, and retinal area. Monnier (1949) confirmed some of the observations of Adrian on specific wave-length effects.

Riggs *et al.* (1949) obtained records of the human action potential for various wave lengths and intensities of stimulating light. Using contact-lens electrodes and a direct-coupled amplifier, they obtained results that agreed with those of Adrian to the extent that two components were observed. The fast one, however, was much less prominent than the one described by Adrian. A spectral-sensitivity curve was computed on the basis of human *b*-wave responses. It was shown to agree fairly well with a psychophysically determined scotopic-sensitivity curve except at the blue end of the spectrum. Blue light was unexpectedly high in its ability to elicit a *b*-wave.

The *x*-wave of Motokawa and the fast component observed by Adrian and others may reflect either a specific response to red light or a more general response of the photopic or cone-receptor visual system. If the latter, there may be some sort of interference that prevents the wave from showing itself optimally on stimulation by light at 555  $m\mu$ , a wave length of light usually associated with the greatest photopic effect.

We may conclude that wave-length specificity is certainly present in the human action potential. There is no clear evidence as yet that color-

discriminating mechanisms of the eye are responsible for any particular component of the action potential, though this possibility must not be overlooked in future research. The form of the *b*-wave does not appear to change with variations in wave length of stimulation.

*Area Effects and the Electroretinogram.* Attempts have been made (Cooper *et al.*, 1933; Adrian, 1946) to stimulate foveal regions of the eye without involving peripheral regions. These attempts have met with little success, however, in arousing a measurable action potential. Fry and Bartley (1935) were able to show why this is true. Working with the rabbit, they found that only an extremely bright flash of light would

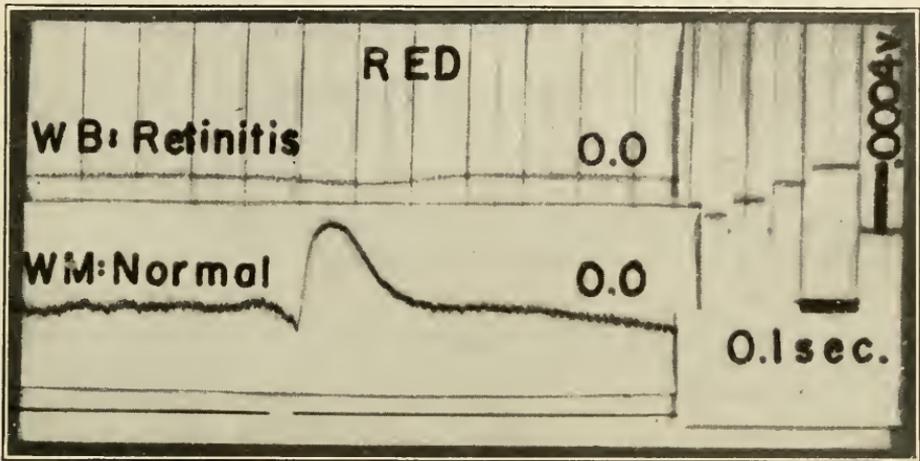


FIG. 13-7. Comparative responses of a normal eye and an eye affected by retinitis pigmentosa.

elicit the action potential when directed upon a small patch of the retina. When two such patches were stimulated alternately, there was no action potential. Hence it was concluded that under these conditions the action potential is not produced by the region directly stimulated but by the relatively large nonfocal area to which light is reflected and scattered within the eyeball.

This result, amply confirmed in experiments on the human eye (Boyn-ton and Riggs, 1951), makes it difficult if not impossible to elicit an action potential from photopic receptors without a large involvement of scotopic ones as well. Scotopic responses may be suppressed to some extent by the use of brief exposures, moderate degrees of light adaptation, and stimuli made up of the longer wave lengths of light.

*Clinical Use of the Electroretinogram.* When the contact-lens electrode was first introduced, the suggestion was made (Riggs, 1941) that clinical cases might be more easily investigated by this means. Karpe (1945, 1948a,b) has reported the results of testing a number of patients with various retinal disorders, and a number of such observations have been

made less systematically in this country by the writer and others. It is notable that some disorders (e.g., retinitis pigmentosa) abolish the electroretinogram even when the degeneration has not progressed very far. This finding suggests the possible use of the electroretinogram as a device for early diagnosis of such disorders. Figure 13-7 presents a comparison between a normal electroretinogram and one obtained from an eye affected by retinitis pigmentosa.

The effects of anoxia upon the action potential of several animals, including man, have been reported by Noell and Chinn (1950; Noell, 1951) as a part of their research on the visual pathways (see later). In general, the *b*-wave of the electroretinogram is depressed relatively soon, so that only the negative PIII component remains after prolonged oxygen deprivation, as in Granit's experiments (1933).

#### CONCLUSIONS WITH REGARD TO THE RETINAL ACTION POTENTIAL

In summary, the action potential has the following features: (1) It is relatively easy to record in most animals and is the only electrical response that is suitable for recording in the human eye. (2) In invertebrates it appears to originate in the visual sensory cells. (3) In vertebrates it contains a number of components, the most prominent of which, the *b*-wave, is thought to originate at some point between the sensory cells and the ganglion cells of the retina. (4) In man the *b*-wave has scotopic characteristics; earlier waves appear to reflect photopic or specific red-receptor activity. (5) The usefulness of the electroretinogram as a tool for exploring particular retinal areas is seriously limited by the necessity of using high intensities and large areas of stimulation. (6) The action potential has certain applications to clinical problems.

#### OPTIC NERVE IMPULSES

It is evident from this discussion that the electroretinogram can scarcely provide the sort of detailed information which is needed for analyzing visual receptor processes. This can be done only by the far more difficult procedure of placing electrodes directly on the separate elements of the retina (sensory cells, retinal neurones, and optic nerve fibers). Again, as in the case of the electroretinogram, the complexity of the vertebrate retina has made it very difficult to obtain clearly differentiated responses from sensory cells alone. More primitive eyes are more easily used for this purpose.

#### VISUAL RESPONSES OF PRIMITIVE EYES

Hartline and Graham (1932) developed an admirable method for studying basic sensory processes in the lateral eye of *Limulus polyphemus*, the horseshoe crab. A small bundle of fibers is dissected free from the

optic nerve and allowed to rest on two recording electrodes, as shown in Fig. 13-1. Stimulation of the eye by light results in a train of nerve spike potentials that are recorded by the use of a suitable amplifier and oscillographic camera. Further dissection may be used to isolate a bundle containing but a single active nerve fiber. A sample of the resulting response to light is shown in Fig. 13-8.

The anatomical evidence is that a fiber of the *Limulus* optic nerve stems directly from an individual sensory cell. Impulses recorded as

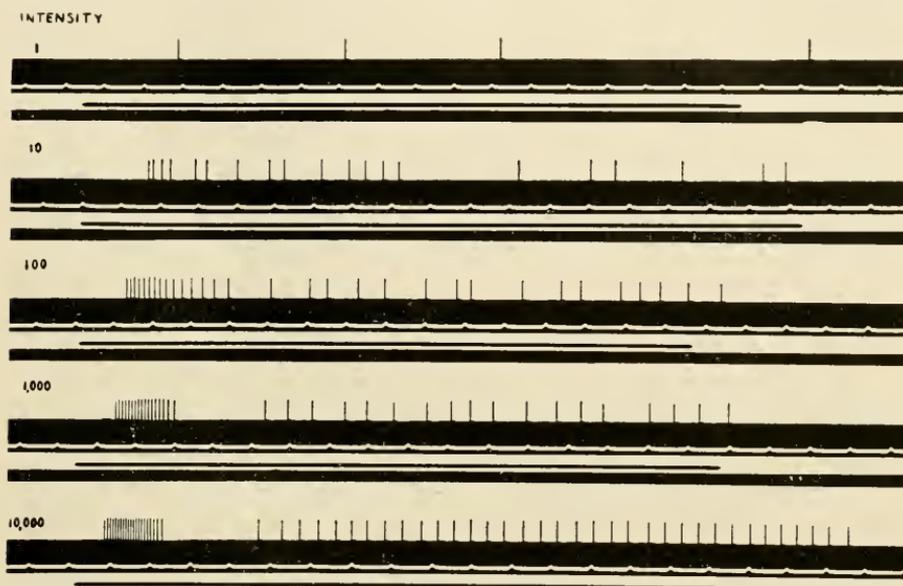


FIG. 13-8. Responses of a single optic nerve fiber of *Limulus*. In each record the vertical spikes represent impulses recorded by electrodes placed as in Fig. 13-1c. The time line has notches every fifth of a second. Duration of the stimulus is denoted by the horizontal black line.

described are accordingly those of a true sensory nerve fiber whose impulses have not traversed any synapse or ganglion cell. Recent experiments (Hartline, 1949) have shown some degree of interaction among units of this sort and have raised the possibility that synaptic connections may exist, though undetected as yet by histological examination.

*Characteristics of the Single-fiber Response.* Impulses recorded in a single fiber show the following characteristics: (1) The first impulse in response to illumination appears after a latent interval whose duration is progressively shorter for higher intensities of stimulation. (2) The frequency of the discharge is high during the first burst of impulses, particularly for high stimulus intensities (see Fig. 13-8). (3) There is a "silent period" during which the impulses come less frequently or cease altogether. (4) A steady discharge begins after this silent period and continues as long as the light remains on. (5) There is not usually an

off-effect. Occasionally, as a result of very strong stimulation, there is a silent interval followed by a short train of impulses after the light goes off. (6) The expected characteristics of single-nerve-fiber responses are observed: the magnitude and form of the spike invariant with the duration, the intensity, the wave length, or other characteristics of the stimulating light.

*Reciprocity of Time and Intensity of Light.* Stimulus duration and intensity are reciprocally related over wide ranges in the arousal of impulses in the *Limulus* optic nerve fiber (Hartline, 1934). Evidently the photochemical reaction within the sense cell is so simple and so accurately represented by the nerve discharge that the Bunsen-Roscoe law ( $I \times t = k$ ) is obeyed. For very short flashes, stimulation occurs entirely during the latent period and has therefore ceased before the nerve response begins to occur. This shows that the latent period is not that of the photochemical reaction but rather that of subsequent processes whose velocity and total extent are precisely governed by the photochemical one. The intensities that are capable of affecting the response of a single ommatidium certainly cover a range of more than six log units.

*Spectral Sensitivity.* Another property related to the photochemical process is that of spectral sensitivity. Graham and Hartline (1935) measured the energy of light, at each of several different spectral locations, which was required to elicit an impulse discharge. They assumed that a given amount of energy must be absorbed by the receptor cell in order for it to discharge an impulse. They were therefore able to compute an absorption spectrum for the photosensitive substance. The resulting data showed a maximal sensitivity for the region between 500 and 550  $m\mu$  and a curve whose form is in general agreement with that of the absorption spectrum of visual purple.

*Dark Adaptation.* The course of dark adaptation in the sensory cells of *Limulus* is surprisingly similar to that of higher animals, including man. This conclusion, first obtained (Hartline, 1928) by the use of the electroretinogram, was substantiated in more detail by later work on the single receptor unit (Hartline and McDonald, 1947).

Dark adaptation may be measured by preexposing the eye to steady illumination and then determining the intensity of a test flash that is just sufficient to evoke a single impulse in the optic nerve fiber. This procedure, analogous to that of determining threshold intensities for the human eye, shows that the critical intensity falls rapidly during the first few seconds after the preadapting light is turned off. The critical intensity then continues to decline at a progressively slower rate for an hour or more under typical conditions.

The dark-adaptation process takes longer after a strong preadapting light than after a weak one. The intensity and duration of preadapting exposure are nearly reciprocal quantities over a wide range. However,

the reciprocal relation does break down for very low intensities or very long exposure times. The results here are consistent with those obtained by Wald and Clark (1937) and Crawford (1946) in the human eye and by Riggs (1937) in the eye of the frog. Prolonged exposure to weak illumination is followed by an inordinately long recovery process in all these experiments. This finding is consistent with the concept of a dual restoration reaction as described by Wald and Clark (1937).

In one other respect the *Limulus* data are of particular importance for the interpretation of the dark-adaptation process. Hartline and McDonald observed in detail the train of impulses following various intensities of test flash at various times in the dark. Their conclusion was that it was not possible to match such a record in the fully dark-adapted eye with any single record in a partially dark-adapted one, even by the appropriate adjustment of stimulus intensities. In other words, it is true even in this primitive eye that qualitative as well as quantitative differences exist in the responses obtained at various stages of the adaptation process. It therefore follows that the "sensitivity" or reciprocal of threshold intensity is only one of several possible indexes of the course of dark adaptation.

*Light Adaptation.* Hartline and McDonald (1947) also studied the adaptation of the *Limulus* receptor element to fixed levels of illumination. They first adapted the eye for 10 min or more to a given level, then interrupted the light and applied a test flash after 1 sec of darkness. The resulting data show that proportionately higher intensities of the test flash must be employed at the higher levels of light adaptation. At each level, however, the curve relating the number of impulses to the logarithm of the intensity of the test flash is similar to each of the other curves, simply displaced along the intensity axis. In other words, the sensory cell maintains a full range of responsiveness at all levels to which it can adjust itself.

Another method of studying light adaptation was used by Riggs and Graham (1940, 1945). A test flash was added to the adapting light at various times after the adapting light was turned on. The effect of the test flash was to cause a momentary increase in the frequency of nerve impulse discharge. Strangely enough, the receptor unit became increasingly responsive to such a test flash during the first minute of light adaptation; after that the effectiveness of the added flash diminished steadily with increasing exposure to the adapting light. A somewhat similar finding in human vision has been reported by Baker (1949).

*Receptor Variability.* There appear to be conditions under which relatively large fluctuations of sensitivity may take place in a sense cell even when a steady photosensory state may be reasonably assumed. One such condition is that of the refractory period in the sense cell (Riggs, 1940). Immediately following the discharge of any impulse in response to steady

illumination of the eye, the photoreceptor unit is found to be relatively incapable of responding to a test flash of light (see Fig. 13-9). The relative refractoriness of the sense cell may persist for half a second or more under some conditions. Such prolonged refractoriness may be characteristic of the mechanism by which the end organ initiates repetitive impulses in the attached nerve fiber.

*On- and Off-responses.* The sea scallop, *Pecten irradians*, was found by Hartline (1938b) to have a retina with two layers of sense cells. Nerve

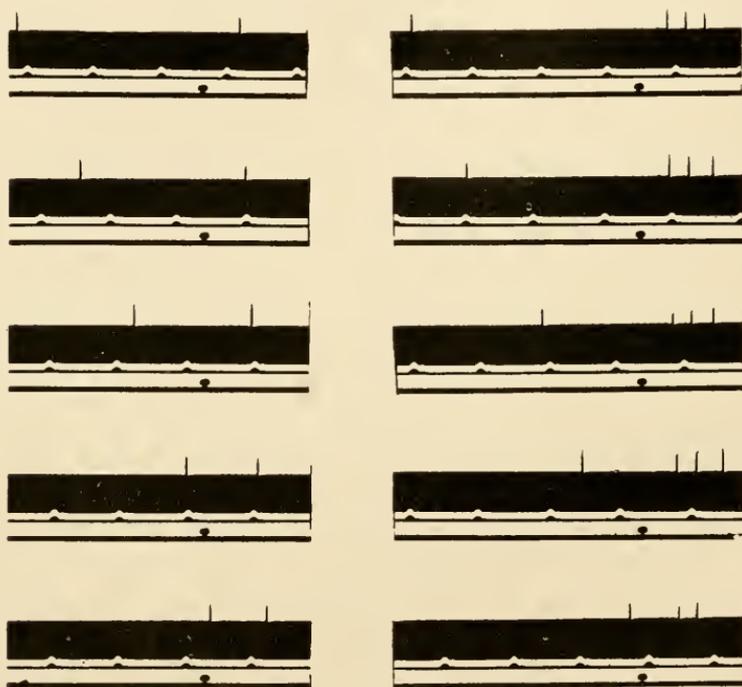


FIG. 13-9. Changes in latency of response as a function of recovery from the discharge of an impulse in the *Limulus* photoreceptor unit. A steady light, denoted by the horizontal black line in each record, has been on for some time and is responsible for the discharge of impulses at infrequent intervals. An added flash of light (denoted by the black dot) elicits a response whose latency depends upon the state of recovery of the sense cell. (Riggs, 1940.)

fibers from the proximal layer respond to the onset of illumination, but those from the distal one respond when the light is turned off. Hartline points out, however, that the neural off-response may originate as such in retinal receptor cells or may instead be developed in synaptic connections with secondary neurones. Unfortunately the histological evidence is not clear as to the presence or absence of such synaptic connections.

#### RESPONSES OF VERTEBRATE EYES

The above discussion of invertebrate eyes was concerned with the responses of primary or secondary neurones. In the typical vertebrate,

as mentioned earlier, the fibers of the optic nerve are those of third-order neurones, the retinal ganglion cells. Responses recorded in them will have been modified by the presence of at least two synapses between them and the primary sense cells of the retina. Horizontal and amacrine cells may introduce facilitation or inhibition. Convergence is such that several receptor cells may supply a single optic nerve fiber.

*Spatial Summation.* Adrian and Matthews (1927a,b, 1928) measured responses in the optic nerve of the eel, *Conger vulgaris*. Both on- and off-effects were observed. Area and intensity were found to be reciprocally related, in the arousal of nerve impulses, for regions up to several

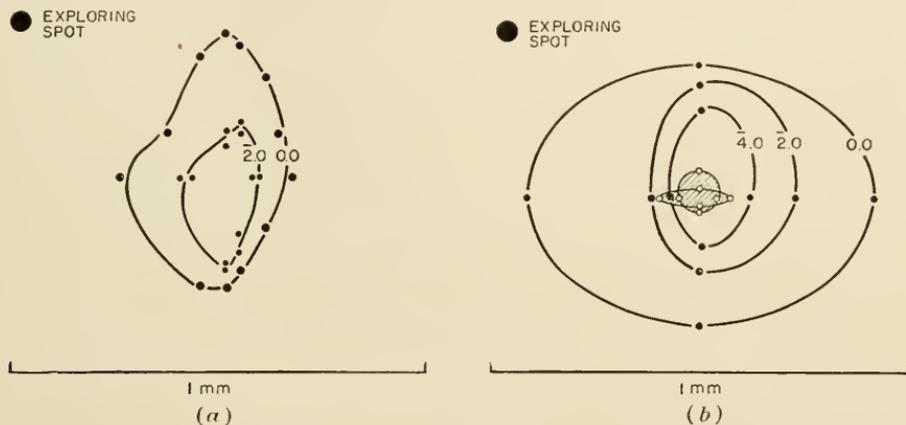


FIG. 13-10. Receptive fields for single vertebrate optic nerve fibers. Contours at various levels of intensity of the exploring spot. Each contour is labeled with the logarithm of the intensity that is just capable of eliciting minimal responses in the fiber (relative to maximum intensity of  $2 \times 10^4$  m-c). In (b) stimulation at high intensity within the shaded region evokes a maintained discharge. Elsewhere, in (a) and (b), discharge was not maintained, however intense the stimulating light. (Hartline, 1940b.)

tenths of a millimeter in diameter on the retina. Spatial summation was increased by the application of strychnine. Since this drug is known to facilitate synaptic transmission, the authors concluded that the summation that they observed was the result of a lateral spread of excitation, carried out for the most part by neural interaction.

The most elegant demonstration of retinal interaction is to be found in Hartline's work (1940a,b,c) on the optic nerve fibers of the frog. In these experiments the eye was excised, and the retina was exposed by removing the anterior half of the eyeball and draining away the vitreous humor. Small bundles of nerve fibers were then dissected free from the anterior surface of the retina. These are third-order neurones making their way from various parts of the retina toward the optic disc, where they go to make up the optic nerve. By painstaking dissection it was sometimes possible to obtain a bundle containing but a single active nerve fiber. A cut end of the bundle was lifted onto a wick electrode.

The indifferent electrode was a second wick in contact with the surface of the retina.

Hartline used a small exploring spot of light to locate the portion of the retina whose stimulation would arouse impulses in the isolated nerve fiber. Typically the sensitive field so located had the appearance shown in Fig. 13-10. It is clear that a wide area is served by this fiber and that a small region at the center of this area is most effectively represented.

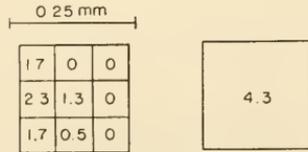


FIG. 13-11. Stimulus area on the retina of a frog. *Left*: frequencies of maintained optic-nerve discharge (single fiber) for each of nine small squares tested individually. *Right*: frequency of discharge in same fiber to stimulation of the entire area covered by these squares. Intensity 300 m-c in all cases. (Hartline, 1940c.)

Further experiments were performed in which the degree of spatial summation was measured for a region within the receptor field. Figure 13-11 illustrates the region so employed, and Fig. 13-12 shows a sample of the resulting responses of the nerve fiber. It is clear that a higher frequency of nerve impulses results from the stimulation of the large area than from stimulation of the most sensitive small patch within it.

The experiments just described show clearly the following: (1) Spatial summation extends over a relatively large area of the eye of a frog. (2) Subliminal excitation of small regions may result in a discharge when several such regions are illuminated together; hence more than one impulse must originate in the

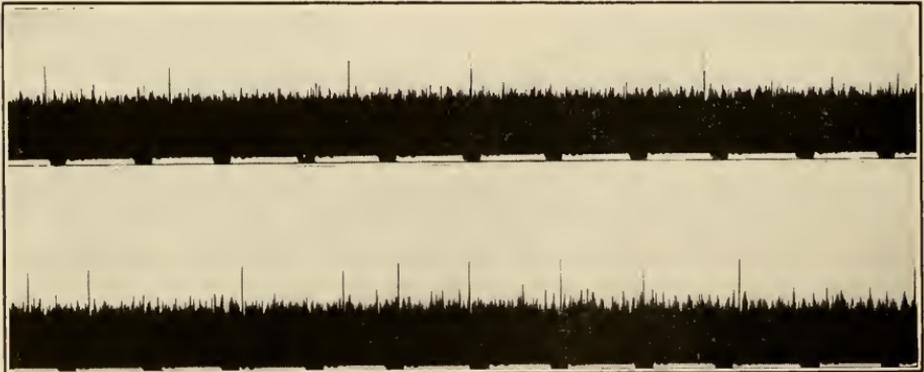


FIG. 13-12. Records showing spatial summation in the arousal of impulses in a single optic nerve fiber of the frog. *Top*: maintained discharge in response to illumination of most effective region (small square labeled 2.3 in Fig. 13-11). *Bottom*: response to illumination of the entire area (large square labeled 4.3 in Fig. 13-11). (Hartline, 1940c.)

converging pathways in order to arouse the final common path, the optic nerve fiber. (3) With very high intensities of stimulation there is a smaller response from a large region of stimulation than from a small one.

(4) Stimulation of the area outside the receptive field of a nerve fiber has no effect on the responses of the fiber. (5) All these effects have been observed under conditions that preclude scattered light, chemical spread, or electrotonic spread as mechanisms for the observed summation.

Hartline's receptive-field experiments have furnished detailed information about the process that is presumably responsible for the extraordinarily great sensitivity of human peripheral vision. Graham, Granit, and others have shown that off-center vision is highly sensitive to low levels of illumination (see Graham, 1934). Convergence of neural pathways has achieved this result at the expense of visual acuity. Central vision, on the other hand, shows higher acuity but fails completely at low levels of illumination. It is known that there is relatively little convergence of pathways within the central fovea of the human eye.

*Specificity in Optic Nerve Fibers.* The law of specific nerve energies states that every impulse is similar to every other impulse within a nerve fiber, so that qualitative differences cannot be directly signaled along a single pathway. Sensory end organs are specifically tuned, however, to mediate sensory qualities such as warmth, cold, or pain. Likewise there are nerve fibers that, like some found in the vagus nerve, have inhibitory rather than excitatory effects.

The vertebrate retina is easily the most complex nervous region outside the central nervous system. As such, it may contain inhibitory as well as excitatory fibers, and its individual receptor cells may respond selectively to separate characteristics of the visual stimulus. The trichromatic theory demands that at least three types of color receptor be present. The duplicity theory asserts the presence of two types of receptor, the rods and the cones. The existence of aftereffects and contrast phenomena suggest, at least, that some sort of inhibitory mechanism may also be important. With these speculations in mind, let us review some of the information that is obtainable from optic nerve fibers of the vertebrate retina.

Hartline (1938a), using his dissection method on the opened eye of the frog, found that only about 20 per cent of the fibers responded, as do those of *Limulus*, with an initial burst followed by a maintained discharge. About 50 per cent showed an initial burst when the light appeared and a final burst after the light went off, with no discharge during steady illumination. The remaining 30 per cent of the fibers showed no response at all to illumination but gave a vigorous and prolonged discharge after the light was turned off. Figure 13-13 shows samples of the three types of response.

On-responses were most vigorous when elicited after a long period of darkness, whereas off-responses were at their best following prolonged illumination of the eye. Off-responses were suppressed by reillumination. The specific character of the response did not change with variations in

temperature, state of adaptation, asphyxia, carbon dioxide, ion imbalance, or type of stimulation. The eyes of certain fish, amphibia, and reptiles all gave essentially similar results.

Granit and his coworkers have used a different technique from that of Hartline for recording the responses of retinal fibers. They have removed the cornea and lens but have done no retinal dissection. Instead they have used a microelectrode (fine platinum wire insulated except at the tip) as their active lead. An indifferent electrode is applied to the back of the eye (see Fig. 13-1). Granit (1947, p. 304) has expressed some

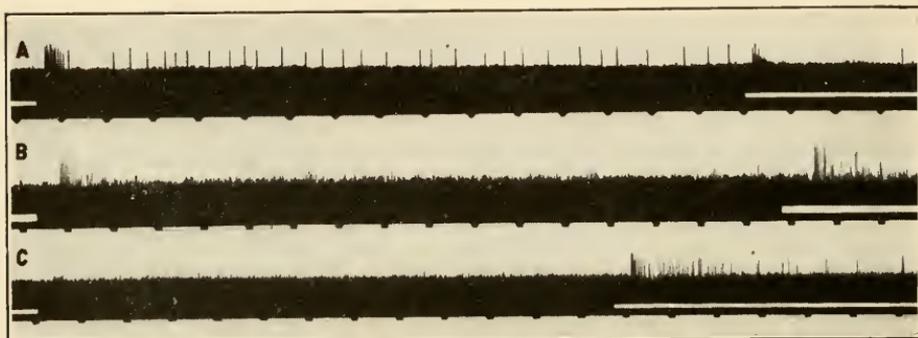


FIG. 13-13. Responses of three types of retinal fiber in the frog. (A) Fiber responding with an initial burst and maintained discharge. (B) Fiber responding to onset and cessation of light. (C) Fiber responding only to cessation of light. In each record the signal marking the duration of the stimulus fills the white line above the time marker. Time is in units of one-fifth of a second. (Hartline, 1938a.)

concern about the possibility of confusing the response of two well-synchronized adjacent fibers with that of a single fiber. He points out that, when the technique is faulty, so that single fibers are not obtained, the only "final common path" in the experiment is the microelectrode itself. He believes that this objection holds generally, however, for all isolation techniques except those in which a single end organ is stimulated.

Some of the principal findings of Granit's laboratory (Granit, 1947, 1950a,b) are the following:

1. Discharge types: Mammalian eyes show the three types of discharge found by Hartline in the frog. The maintained-discharge type, the on-elements, predominate in the guinea pig, whose retinal receptors are mostly rods. In the cat retina they have the spectral sensitivity characteristics to be expected of responses to the bleaching of visual purple in rod receptors. The off-elements are more numerous in cone retinas. Granit associates these off-responses with the *d*-wave (off-response) of the electroretinogram. The on-off elements are characteristic of cones, though both they and the off-elements may represent some rods as well.

2. The off/on ratio: This is the ratio between stimulus thresholds for

off- and on-responses. It has been found to vary from 0.001 to 10,000 in the cat. There is some evidence for a relation between the off/on ratio and wave-length sensitivity. Granit believes that the on-off elements are most likely to be the basis for color discrimination in the cat.

3. "Dominator" elements: In the dark-adapted cat the spectral sensitivity measured for a retinal element often resembles that of visual-purple absorption. Such an element is known as a "scotopic dominator." Certain fish have a visual-violet system, and in them the scotopic dominator element is found to have a spectral-sensitivity curve with the higher wave-length maximum characteristic of that substance. After light adaptation most elements in an eye having cone vision become "photopic dominators" with a maximum sensitivity shifted to a higher wave length (Purkinje shift). Fish that have a visual-violet system shift to a photopic dominator at a still higher wave length. No scotopic dominator was found in the cone eye of the snake, and no photopic one was found in the rat or guinea pig, whose receptors are mostly rods.

4. "Modulator" elements: Some light-adapted elements of the frog, rat, guinea pig, and snake showed narrower spectral-sensitivity curves than those described as dominators. These elements were individually tuned to respond to particular wave lengths of light and were named "modulators" on the assumption that they were the basis for qualitative discrimination of color. In the cat it was necessary to use an indirect method to find such modulators, since in only a single experiment was a modulator ever found by the direct method in this eye. The indirect procedure used in finding modulators in the cat was, first, to light-adapt the eye selectively by the use of red, green, or blue light obtained through Ilford spectral filters. Then the spectral sensitivity function was determined for any given retinal element. If this function resembled that of the scotopic dominator, Granit concluded that only receptors containing visual purple were connected to the fiber being studied. If, on the contrary, the spectral-sensitivity curve departed from that of the scotopic dominator, the conclusion was drawn that cone receptor elements were present along with rod receptors in the region served by the fiber in question. This situation is shown in Fig. 13-14. Here it is shown that a colored adapting light has changed the spectral sensitivity function of one particular element from that shown in curve  $P$  to that in curve  $u$ . Curve  $p$  is the result that might have been expected for light-adapted rods alone. The fact that  $u$  shows relatively less diminution in the region near  $550\text{ m}\mu$  is interpreted to mean that one or more cone elements are present whose maximum sensitivity lies in this region. These cone elements are assumed to be modulators whose response curves are obtained by the procedure of subtracting curve  $p$  from curve  $u$ . Figure 13-15 shows some examples of the resulting modulator curves for the cat. These are averages obtained in the course of several experiments. Granit

concludes that he has demonstrated a basically trichromatic mechanism for color discrimination in the cat.<sup>3</sup>

On the basis of the work just described, Granit assumes that in most cases his microelectrode makes contact with a retinal third-order neurone that is served by several receptor elements. When rod cells alone are

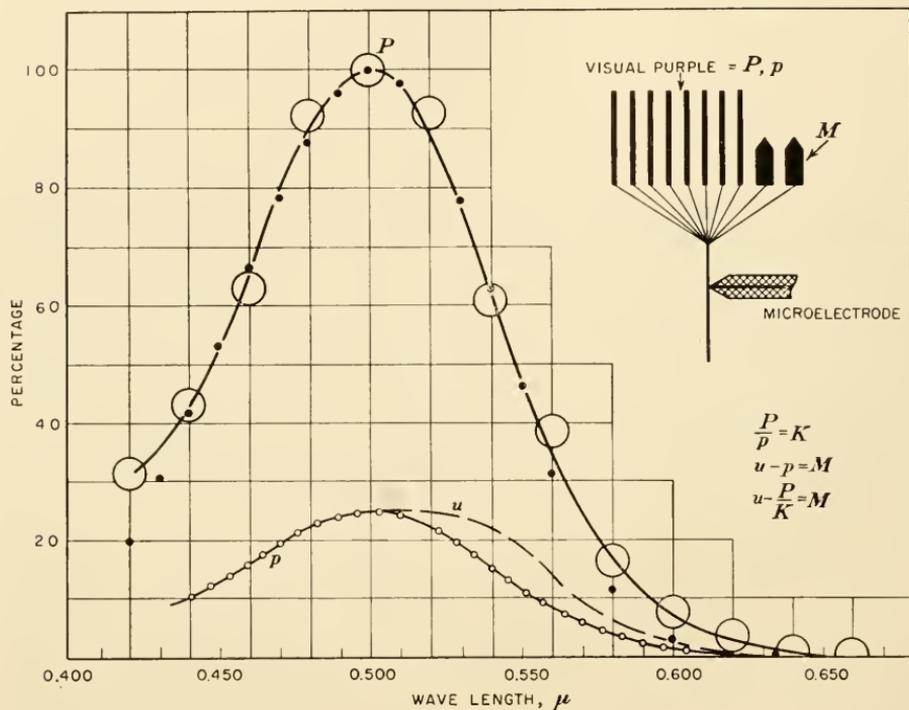


FIG. 13-14. Spectral-sensitivity curves for the cat, as analyzed by Granit (1947, p. 311). Ordinate: relative sensitivity, evaluated for an equal quantum intensity spectrum. Abscissa: wave length of stimulating light. Curve *P* is from corrected values for visual-purple absorption. The other curves are those which would be obtained from receptors containing visual purple alone (curve *p*) and from these receptors in combination with modulator (*M*) receptors (curve *u*) after selective adaptation to filtered light (red, green, or blue filter).

involved, typical scotopic dominator characteristics are revealed by the responses of the nerve fiber. A mixture of rods and cones permits the shift from a scotopic to a photopic dominator as the level of illumination is raised. The cone cells differ in their wave-length sensitivities, but the presence of several different ones within the region of convergence on the

<sup>3</sup> Behavioral evidence has not revealed that cats are capable of color discrimination. Although negative evidence of this sort is never conclusive (Granit, 1950b), it may be pointed out that only by selective adaptation have the photopic modulators revealed themselves. If a cat receives no more information from its optic nerve fibers than is recorded in these experiments, the animal must be color-blind most of the time.

retinal fiber is likely to make it a photopic dominator that has a rather broad spectral range of sensitivity. A predominance of red-sensitive cones will, however, render the fiber particularly responsive to red; in this case it is called a "red modulator." Green and blue modulators of various sorts are also found by the indirect means of selective adaptation just described.

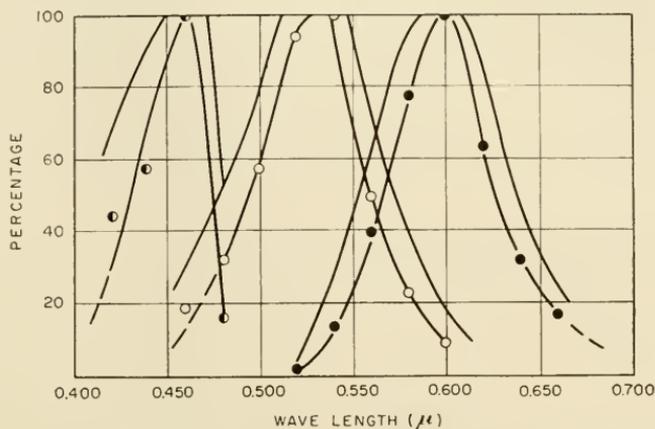


FIG. 13-15. Average modulator sensitivity curves of the cat, obtained by selective adaptation (*Granit, 1947, p. 312*). Filled circles: red modulator curves resulting from selective adaptation to blue or green light. Open circles: green modulator curves obtained after adaptation to red or green light. Half-filled circles: blue modulator curves obtained after adaptation to red or green light.

#### CONCLUSIONS WITH REGARD TO THE RESPONSES OF OPTIC NERVE FIBERS

The following conclusions may be drawn with regard to the response of optic nerve fibers: (1) The optic nerves of some primitive eyes appear to be made up of true sensory fibers without synapses. They reveal the responses of the end organs, uncomplicated by the effects of convergence or interaction. (2) Light and dark adaptation in single sensory cells of invertebrates are similar in detail to these processes as observed in higher animals, including man. (3) Some variability in the responsiveness of a single end organ is associated with a recovery process following the discharge of each impulse. Random variability is also present. (4) Vertebrate optic nerve fibers are those of third-order neurones, the retinal ganglion cells. Three fundamental types of response to light are exhibited by such fibers (on-, on-off-, and off-responses). (5) Each fiber typically serves a relatively large receptive field of the retina. Spatial summation is readily demonstrated within this area. (6) Specific color sensitivity appears to characterize some of the vertebrate retinal fibers, but only very indirectly is it possible to infer the color-responding properties of the corresponding receptor cells.

RESPONSES OF THE OPTIC TRACT, GENICULATE BODY,  
AND CORTEX

We now leave the retina and turn our attention to events in the optic pathways. Pioneer investigations have been made by Wang and Lu (1936a,b, 1937), Bishop and O'Leary (1938, 1942), Bartley (1941), Marshall and Talbot (1942), Adrian (1946), and Noell (1951), among others. These investigations have in common the purpose to follow, by electrical recording, the course of visual excitation from the eye to the cortical projection areas. It is immediately apparent that in this form of recording there is more variability and less precise localization than is characteristic of single-fiber preparations. Some of the principal findings are as follows:

1. *Optic-tract Potentials.* In mammals these typically show several peaks, including two large ones (the "early-on" and "late-on" waves) with latencies of about 15 and 50 msec, respectively, after the beginning of stimulation by light. Off-responses may also be complex, especially after prolonged stimulation by light. The latency of the largest off-response may lie between 20 and 50 msec. Mammalian optic-tract potentials often appear to have latencies lower than the latency of the action potential, and the action potential often fails to show any off-effect similar to that of the optic tract. The two on-waves differ markedly with respect to the effects of stimulus intensity; the late on-wave has a lower absolute threshold, but it is diminished or even abolished at high stimulus intensities.

2. *Geniculate Potentials.* Bishop and O'Leary (1942) have made an analysis of the responses that may be picked up in the vicinity of the dorsal nucleus of the lateral geniculate in the cat. These are more spiked, with less prominent separate waves. Furthermore the geniculate responses are more variable from time to time than are the optic-tract potentials, and their wave form is dependent on the exact position of the recording electrodes.

3. *Cortical Potentials.* Electrodes on the occipital cortex reveal spontaneous activity including, most prominently, the  $\alpha$  rhythm of 3-12 waves per second in various animals. In response to flashes of light (or to electrical stimulation) several response waves are seen, sometimes lasting for a second or more. The form of the response shows great variability, even when elicited under uniform experimental conditions. It is difficult to assign any one component of the cortical response to any definite location in the cortex.

Some of the experimental factors that influence cortical response potentials are the following:

1. Strychnine initially causes an increase in the first portion of the response, then raises the threshold of stimulation and causes a diminution

in the slower components. Still later the  $\alpha$  activity is reduced or abolished by strychnine, and spontaneous diphasic spikes begin to appear in trains. During and immediately after these spikes the cortex is not excitable by optic nerve impulses. It is therefore concluded that the spontaneous activity and response activity are similar with respect to their cellular origin and functional characteristics (Bartley *et al.*, 1937).

2. Area, duration, and intensity of stimulation all have the power to influence the implicit time (time to peak) of the first cortical-response wave. Bartley (1941) has noted that an increase in any or all of these factors leads to a reduction in response time. With respect to the area factor, he found evidence that two effects were operating independently—stray light and neural interaction.

3. The cortical  $\alpha$  rhythm and cortical-response activity were found by Bartley (1941) to be closely interrelated. The arrival of optic nerve impulses might produce any effect ranging from abolition of existing  $\alpha$ -waves to the initiation of these waves if they were originally absent. These effects chiefly depended upon the frequency and phase relations between the spontaneous and evoked potential waves.

4. Localization on the cortex of two separate visual areas has been studied by Thompson *et al.* (1950). They have mapped the areas representing the various portions of the retinal fields. The wave forms of responses from the two areas are similar.

5. The wave form of the cortical response has been analyzed by Chang and Kaada (1950). These investigators noted six component deflections in the typical cortical response to electrical stimulation of the optic nerve in the cat. They attributed the earliest component to the optic-nerve volley itself, the next three to separate geniculocortical connections, the fifth to intracortical neurones, and the sixth to cortical internuncial cells. They also considered the possibility that the three geniculocortical paths may relate to a three-color visual mechanism.

#### THE SEQUENCE OF EVENTS FROM RETINA TO CORTEX

Of particular interest are investigations in which an attempt is made to relate events occurring in a lower station to those of a higher one in the visual pathways. Adrian (1946), for example, has made simultaneous recordings of the retinal action potential and the optic nerve responses in the rabbit and cat. He concludes that, although there are many points of disagreement between the two forms of recordings, “. . . the response of the eyeball seems to be a reasonable guide to the performance of the receptor mechanisms.” Adrian has also obtained simultaneous records of the action potential of the retina and the responses from the striate area of the brain of the monkey (*ibid.*, p. 35). He has found that blue light, which is very effective in arousing the action potential, is much less so for arousing a cortical response. Red light has the opposite charac-

teristics. It therefore appears that some photopic events elicit a relatively greater response in the cortex than in the retinal action potential (see Fig. 13-16).

Further evidence for this conclusion is provided by Adrian's experiments (1946) on the optic nerve discharges in the cat, rabbit, and guinea pig. Light adaptation greatly reduces the retinal action potential but does not interfere very much with optic nerve impulses. The action potential shows, moreover, a duality of response in which the early component is assumed to be photopic, whereas the later one (*b*-wave) is scotopic. No such duality appears in the records from optic nerve or cortex. Adrian concludes: "It is presumably the function of the eye to furnish

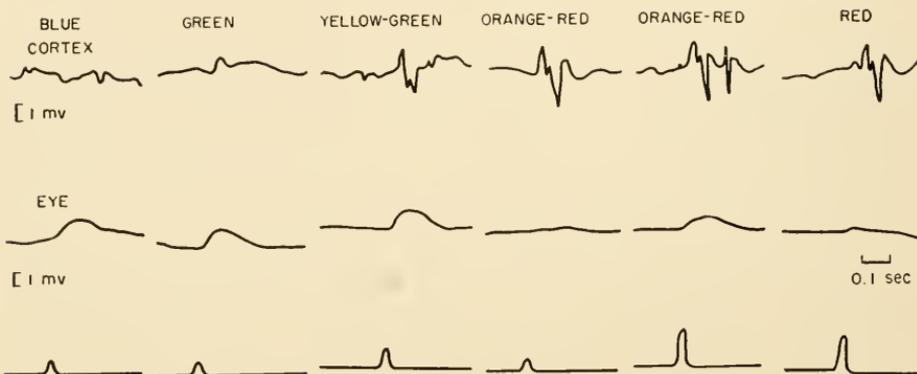


FIG. 13-16. Comparative responses of the cortex (striate area) and the eye (electroretinogram) of the monkey. (Adrian, 1946.)

the brain with a coherent account of visual events, and, although it may employ two kinds of receptors, it has abundant synaptic connections for welding their twin messages into one."

A notable contribution to our knowledge of visual function has been made by Noell and Chinn (1950; Noell, 1951). These investigators have studied the action potential, the activity of retinal fibers, and responses within the optic tract, geniculate body, and cortex. They have worked with man, monkey, cat, rabbit, pigeon, turtle, and frog. Some of their principal findings are the following: (1) Retinal metabolism is based upon both glycolysis and respiration. Respiration predominates in lower vertebrates, glycolysis in mammals. (2) Separate processes within a given retina may be relatively more dependent upon respiration or upon glycolysis. For example, the *a*-wave of the rabbit is early affected by anoxia, thus indicating a dependence upon respiration, whereas the *b*-wave is easily abolished by the presence of sodium iodoacetate, a poison that prevents glycolysis. (3) In the cat, monkey, and man the *b*-wave is more susceptible to anoxia than is the *a*-wave. However, a small *b*-wave is still present at the time when optic-tract potentials have dropped out completely in the rabbit (see Fig. 13-17). Furthermore the human

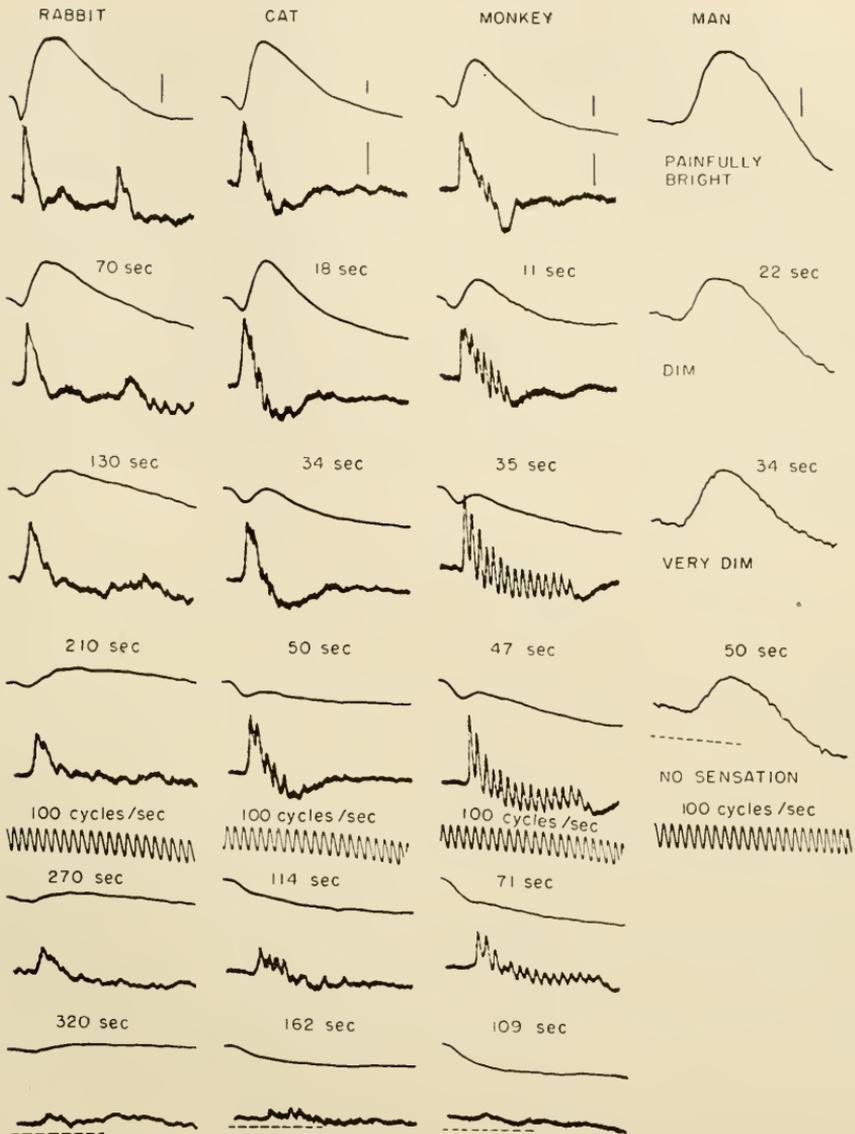


FIG. 13-17. Electroretinogram and optic-tract response during asphyxia of the eye. (Noell, 1951.)

*b*-wave has not completely disappeared at the moment when vision has been temporarily destroyed by reducing the flow of blood to the eyeball. It is concluded that, with retinal asphyxia, human vision fails at the moment when the ganglion cells are inactivated. The *b*-wave originates presumably in the sense cells or bipolars, and these elements are apparently more resistant to asphyxia than are the ganglion cells. (4) In harmony with the above conclusion is the finding that some activity may be detected in first- or second-order neurones of the rabbit after the third-

order neurones (ganglion cells) have ceased to function. This observation was made by the use of a microelectrode inserted into the retina of a rabbit in such a way as to reach the bipolar cell region. (5) Electrical stimulation of the optic tract reveals that conduction is possible even after responses to photic stimulation are lost. This suggests that retinal elements are inactivated sooner than optic nerve fibers are during anoxia. (6) In the geniculate region of the rabbit, responses of the postsynaptic elements are lost in the early stages of anoxia at a time when optic-tract activity has not yet been seriously impaired. (7) The striate area of the cortex is most susceptible to anoxia. The initial effect is to fuse the normally small and uncoordinated deflections into a monophasic wave of much greater size. This effect, together with the subsequent disappearance of all cortical responsiveness, leads to the conclusion that mechanisms of summation and inhibition are among the first to be affected by anoxia.

## ELECTRICAL STIMULATION OF THE VISUAL SYSTEM

### VISUAL EFFECTS OF ELECTRICAL STIMULATION

The visual system is of course capable of being aroused by electrical stimulation at any point from the retinal receptors to the centers in the brain. Variations in the location and intensity of this inadequate stimulation produce concomitant changes in the apparent location, brightness, and hue of the resulting "phosphenes." It has also been reported that the brightness and hue are influenced by the polarity of the stimulating current. Light blue phosphenes are commonly reported for stimulation in which an electrode placed in contact with the front of the eye is positive, whereas dark- or reddish-yellow ones result from current flowing in the opposite direction. Alternating current produces visual flicker, and it is even possible to induce flicker by placing the head within a strong magnetic field of alternating polarity. For the most part no attempt has been made to locate the site of action of any of these subjective effects.

### PHYSIOLOGICAL EFFECTS

Physiological studies have also been made of responses to electrical stimulation. Bishop (1933) studied the speed of conduction along fibers in the optic nerves of the frog and the rabbit. He concluded that there were three fiber groups. He speculated on the possibility that the fastest of these may perhaps be associated with spatial form discrimination, leaving the slower ones to mediate brightness.

Following the pioneer studies of Waller (1900), Granit and Helme (1939) undertook to study the changes that electrical stimulation may cause in the wave form of the electroretinogram. They reported that both negative and positive components of the response to light (PII and

PIII in Granit's analysis) were enhanced by cathodal stimulation of the interior of the eye, but that both were depressed by currents of opposite polarity. This they took to mean that PII and PIII are components whose negative and positive signs are inherent within the underlying structures. Their antagonistic properties are thus not to be considered as resulting from opposite orientations with respect to the electrodes.

#### POLARIZATION OF THE EYEBALL

An interesting development is experimentation in which prolonged polarizing currents have been applied to the eye. One application of this technique has been to study the effects of such polarizing currents upon human visual sensitivity. Kravkov and Galochkina (1947) have performed experiments in which anodal stimulation of the eyelids (with the cathode in the subject's hand) appears to lower the absolute threshold for blue-green light while raising the threshold for orange and red regions of the spectrum. Cathodal stimulation has opposite effects. Dark adaptation is also said to be significantly influenced both during and after electrical polarization of the eyeball, and again opposite effects are obtained with opposite polarities. The authors attribute these effects to changes in the relative concentrations of potassium and calcium ions within the retina, supporting this conclusion with experiments in which these concentrations were altered by means of iontophoresis. Sensitivity to green light was raised by calcium and lowered by potassium.

#### POLARIZATION OF INDIVIDUAL RETINAL UNITS

A second application of polarizing currents has been to study their differential effects upon the responses of individual retinal units. Gernandt and Granit (1947) applied microelectrodes to the nasal retina of the cat and listened, with the aid of an amplifier and loud-speaker, to the responses of small units of the retina (presumably from ganglion cells or their fibers). Electrical polarization was through electrodes on the nasal and temporal surfaces of the eyeball. When the nasal electrode is the cathode, the pure on-elements respond to the onset of the electrical current; the pure off-elements respond to the cessation of the current; and the on-off-elements are mixed, some responding to the onset and some to the cessation of the current. When the nasal electrode is the anode, however, the normal functions are upset in that on-elements respond to the cessation of the current and off-elements to its onset. On-off-elements again respond to either onset or cessation, but in their case the situation is greatly complicated by wide variations from one element to another in relative sensitivity to cessation or onset of light. Some of the on-off-elements are highly off-sensitive, and these particular elements behave like off-elements in responding readily to cessation of cathodal stimulation. The on-sensitive elements (low off/on ratios for stimulation

by light) for the most part acted like pure on-elements, responding most readily to the onset of cathodal stimulation. A minority of these, however, gave a readier on-response to anodal stimulation. One generalization, applicable to the electrical stimulation of any on-off-element, was that on- and off-responses were elicited by opposite directions of current. The authors suggest, on the basis of these experiments, that the on- and off-components are unrelated; that on-components arise from rods, whereas off-components arise from cones; and that the various components are tied in with the "element" by means of direct and indirect neural paths, including horizontal and amacrine cells of the retina. They also consider that on-responses are simple and direct, whereas off-responses indicate the predominance of an inhibitory process based on the horizontal or associational neurones.

Electrical polarization has also been studied for its effects upon the responsiveness of retinal elements to stimulation by light. Gernandt (1947) reports that pure on-elements and most of the cathodal on-off-elements are little affected by polarization. Other on-off-elements and off-elements are markedly affected, however, and the on- and off-components may be quite independently altered by the presence of the polarizing current. Specifically, spectral sensitivity was studied in the presence of electrical polarization. The results on the retinal elements of cats bear some resemblance to the findings of Kravkov and Galochkina on changes in spectral sensitivity produced by polarization in the human eye. Cathodal and anodal polarization, for example, were shown to have opposite effects upon the thresholds for stimulation by spectral lights. Although this principle holds for all the basic types of element (on-, off-, and on-off-elements), it is the on-off-elements that again are of greatest interest. For them it is found that the on- and off-components are differentially affected by polarization. The most common spectral locations for marked changes in threshold due to polarization were found to lie at 470, 520, 570, and 600  $m\mu$ . The changes at 600  $m\mu$  are particularly noticeable. Furthermore it is often found that the on- and off-components of the same element are found in "contrasting" regions of the spectrum.

These observations are typical of those made by Granit and his coworkers on the effects of polarization upon sensitivity to light. The interpretation that these authors give is as follows: On-elements behave like visual rods in being relatively more sensitive to short wave lengths of light. Their responsiveness is fairly independent of horizontal conduction paths and hence little affected by electrical polarization. Off-elements and on-off-elements, on the other hand, have partly photopic characteristics. Their responsiveness is much influenced by horizontal conduction and hence by electrical polarization. The coexistence of on- and off-components within the same retinal element, often affected

by "contrasting" spectral stimuli, is taken as evidence for color-contrast effects occurring at a retinal level. Even in the cat, then, it appears that a peripheral mechanism exists for simultaneous contrast and after-image phenomena, since the on-effect for one color may be similar to the off-effect for another. Finally the specific enhancements of certain spectral regions by polarization are taken to mean that the cat is also in possession of a peripheral mechanism for wave-length discrimination. This mechanism is presumably based upon facilitation and inhibition in the horizontal pathways of the retina.

#### EFFECTS OF LIGHT ON ELECTRICAL EXCITABILITY

In the experiments just mentioned the effects of electrical stimulation were studied by the use of test flashes of light. The opposite type of experiment may also be performed, namely, the use of test stimuli of an electrical nature to study the effects of visual stimulation by light. Motokawa (1949a,b,c,d, 1950) has carried out a series of studies of this nature on human subjects. His usual procedure is to stimulate the eye with a 2-sec flash of light. Then, after varying intervals of time in the dark, a test stimulus consisting of direct current is applied for 0.1 sec with the aid of large electrodes on either side of the eye. A study is made of the intensity of electrical stimulation necessary to arouse a just noticeable phosphene or visual effect.

It is found that, as a result of the flash of light, electrical sensitivity is enhanced. This enhancement builds up to a maximum value within a few seconds after the flash of light and thereafter declines fairly rapidly. The interval of time from the disappearance of the light to the moment of maximum electrical sensitivity is called by Motokawa the "crest time." This crest time is found to be relatively independent of the intensity of light used in the stimulating flash. It does depend, however, upon the wave length of the light. Specifically the crest time decreases from about 3 sec at a wave length of 420  $m\mu$  to about 1 sec at 670  $m\mu$ . Motokawa relates the form of this function to that of a psychophysical curve of wave-length discrimination and draws the conclusion that "the physiological basis for hue discrimination lies in the periphery, as has been assumed in the theories of color vision."

In further experiments Motokawa has found that the degree of enhancement of electrical excitability increases with the logarithm of the intensity of the preceding flash of light in a manner suggestive of the usual critical-flicker-frequency curves. Enhancement also increases as the flash is moved in from the periphery to the fovea. Furthermore it is possible to determine the degree of enhancement as a function of the wave length of the stimulating light. When this is done, using an interval of 1 sec from the flash to the electrical test stimulus, the resulting curve looks

like the classical red "fundamental sensation curve" of König. Similarly an interval of 2 sec yields a curve similar to the fundamental green, and 3 sec yields the fundamental blue curve.

Motokawa has applied his new technique to the exploration of a number of other visual functions. Color blindness, for example, was revealed by an absence of the red function for a protanope and of the green one for a deuteranope. In the periphery an independent yellow function was found for normal trichromatic vision.

Contrast effects have also been studied by Motokawa. The most elaborate experiments of this kind were some in which various geometrical figures were introduced with yellow light in the form of line drawings on a screen. After a figure was exposed, a small standard patch of white light was applied at a point near the boundary of the original figure. Finally an electrical stimulus was presented at an interval of 1.5 sec after the standard patch. The electrical threshold was found to vary with the characteristics of the geometrical figure. In particular, it was found that the electrical threshold varied systematically with the nearness of the standard flash to the boundary of the figure. This is called the "inductive effect" of the boundary line.

The inductive effects have been explored for regions around the boundaries of squares, circles, Landolt rings, vernier displacement figures, broken lines, and the classical figures for demonstrating visual illusions. Briefly the results may be summarized as follows: Any region of discontinuity or sharpness of contour yields a steep gradient of induction for the region around it. Thus broken lines have a stronger inductive effect than solid lines do, and the gap in vernier or Landolt objects is accentuated in the region surrounding it. Visual illusions are consistent with the inductive effects that they appear to elicit.

Motokawa concludes that he has demonstrated a direct physiological basis for perceptual phenomena that have long been described subjectively by gestalt psychologists and others. One may certainly question this interpretation on the grounds that the electrical thresholds themselves are subjectively determined, being based upon the appearances of phosphenes as reported by the observer. Certainly the most basic of these very provocative experiments merit repetition in other laboratories. Only when there is a more thorough groundwork of facts on the process of enhancement will we be in a position to interpret the findings as they apply to such complicated functions as color discrimination and visual illusions.

#### SUMMARY AND CONCLUSIONS

1. Electrical phenomena in vision include the stimulation and the recording of neural responses at any point within a visual system.
2. There is a resting potential that, in the vertebrate eye, amounts to

a difference of several millivolts between the (positive) cornea and the (negative) fundus of the eyeball.

3. In response to light the retina develops an action-potential wave, known as the "electroretinogram," whose components have been rather extensively studied in many animals, including man. It seems likely that the vertebrate action-potential wave arises primarily in the sensory cells or bipolar cells of the retina, though no direct evidence for this is yet available.

4. The responses of single optic nerve fibers, in certain invertebrates, provide evidence for the properties of visual sensory cells. Many important visual processes, such as light and dark adaptation, appear to depend primarily upon events within the sensory cell.

5. Optic nerve fibers in vertebrates are those of third-order neurones which display relatively complex responses. There are responses to both the onset and cessation of light. The basis for specific wave-length discrimination is found in many animals. Evidence of spatial and temporal summation and inhibition is also found.

6. Visual centers at the levels of the geniculate body and cortex exhibit increasingly variable responses whose wave form is complex. It is the neurones within these centers which are most easily influenced by drugs and anoxia.

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## CHAPTER 14

### Invertebrate Photoreceptors

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*Photosensory structures. Summary. The photosensory mechanism. Note. References.*

Information concerning the light receptors of invertebrate animals has come from behavior studies, anatomical investigations, and a relatively small number of workers who have made electrical recordings of impulses in nerve fibers serving sensory mechanisms. Commonly the reactions of an animal to light have led to a more complete understanding of the photosensory basis for which structural features can be found. In all metazoans, however, the nervous system interpreting the impulses from a sensory cell is at least as important in determining behavior as are the anatomical aspects of the photoreceptors themselves.

At its simplest, an organism can discern the difference between being in the light and being in the dark. Since light and dark are relative terms, it is not surprising that most such organisms can distinguish between light intensities and that many of them react visibly when the intensity is altered abruptly. All of them respond differently according to the distribution of energy among the wave lengths of the stimulating light. This is a measure of their spectral sensitivity.

A higher degree of organization in both sense organs and nervous system allows an animal to learn the direction from which a beam of light comes, and hence also to detect any change in direction. The organization may consist merely in differential sensitivity within the cell, or it may be assisted by shadow-casting pigment masses or light-concentrating lens systems. In a metazoan the ability to discriminate between different directions of illumination usually rests on the facts that numbers of sensory structures are present, that the various units show individual polarity in reacting to a light stimulus, and that the units are oriented in a number of different directions. Hence a beam of light stimulates the photosensory units differentially. Any alteration in the direction from which light comes involves a change in the degree of stimulation of the many receptors—a reduction in intensity for some, an increase in inten-

sity for others. This combined alteration the central nervous system may interpret as a change in the direction from which the light comes. No special organ is required (Fig. 14-1), although such localization of these functions is common (Fig. 14-2).

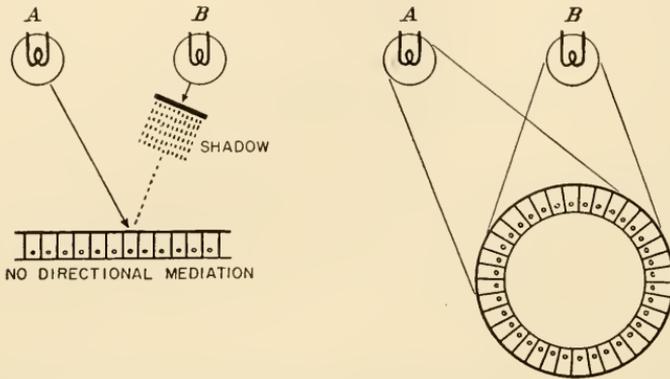


FIG. 14-1. In a flat animal the direction from which light comes cannot be interpreted by a simple integument, but in a cylindrical organism the source of a light can be located because the body itself casts a shadow that can be identified by dermal sensitivity. (Modified from Nagel, 1894.)

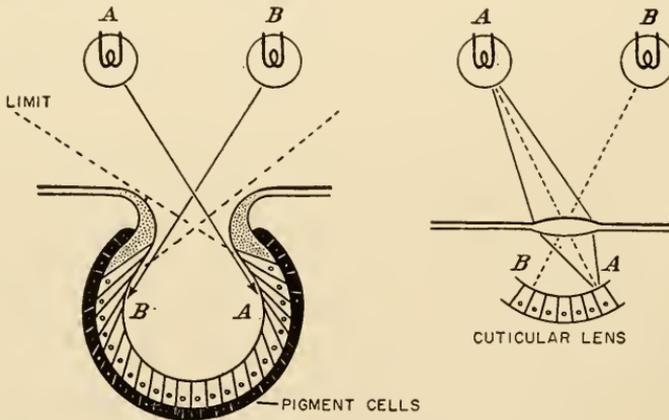


FIG. 14-2. Simple modifications allow a local sensitivity to be used in locating a light source. A pigment cup with a narrow opening limits the light rays. A cuticular lens may provide a brighter spot on a sensory layer a short distance below the body surface. (Modified from Nagel, 1894.)

Structural features that limit the solid angle of surrounding space from which light can stimulate any given unit of a compound photoreceptor, plus an increase in the sensitivity of each unit, may allow an organism to appreciate the distribution of light and dark in its surroundings. If the contrast between an object and the general background is sufficient for the photosensory mechanism to detect it, movement of the object from one place to another may be a source of visual stimulation. The mini-

imum angular size of object which may be followed or used as a visual cue of movement is a measure of the acuity of the system—the resolving power of the mosaic of separate trains of impulses reaching the central nervous system. The accuracy with which an organism can respond to a definite shape of object in its environment is a measure of its appreciation of and reaction to certain forms and outlines and may demonstrate estimation of distance as well as of direction.

In photosensory mechanisms that are particularly highly developed in relation to daytime activities, the ability to distinguish between different parts of the light spectrum may be present—true color vision as contrasted with mere differentiation in terms of brightness. Finally, in the color-discriminating and non-color-discriminating eye, as recently as 1950 an ability has been demonstrated whereby the plane of polarization of sky light is evaluated and used by the organism in orienting its movements.

The structural details in the invertebrate phyla follow no single broad phylogenetic sequence. Analogous mechanisms have developed in group after group. Homologies may be traced within classes. With so very many types of body architecture, the range of photosensory mechanisms might be expected to exceed its actual limits. The largest body of information available concerns the compound eyes of crustaceans and insects, but the widely scattered literature includes data also on a wide variety of other light-sensitive structures.

Since light sensitivity implies radiant energy trapped and the photochemical bleaching of visual pigments is familiar among the vertebrates, a search has been made for corresponding materials among invertebrate receptors. Hensen (1878) reported a pale purplish pigment, bleaching on exposure to light, in the eyes of the scallop *Pecten*. Krukenberg (1882) extracted seemingly similar materials from the cephalopod retina and from the eyes of various slugs and snails and presented spectral-absorption curves for these extracts and for others from earthworm, crayfish, cockroach, and water beetle (*Hydrophilus*). Most of these claims have not been supported by further work.

The cephalopod eye, being much larger and of a camera type, like the eyes of vertebrates, has offered material for investigation of photosensitive pigments. Hess (1902) reported a change in color of the squid retina when dark-adapted specimens were brought into light; he cited similar but less obvious changes in the cephalopods *Sepia* and *Eledone*. Escher-Devières *et al.* (1938) obtained spectral-absorption curves for the retinal pigment of *Eledone*. The pigment seemed similar in action to rhodopsin in the vertebrate eye (Wald's data, 1941), and no chemical difference was detected between the retinal pigments of *Eledone* and *Octopus*. Wald noted that the deep purple color of the squid retina was photostable and suggested that it might be a melanoid. He tested these retinas for vitamin A<sub>1</sub> and for retinene<sub>1</sub> and concluded that, since the vitamin content

did not alter with illumination, it was not involved in the fundamental chemical equilibria. Eyes of the decapod crabs *Carcinus* and *Uca* failed to yield any traces of retinene but contained high concentrations of vitamin A<sub>1</sub>. Bliss (1943, 1946) found that eye extracts from squid, the crab *Callinectes*, and the xiphosuran *Limulus* were photostable until treated with dilute formaldehyde but then bleached to release large quantities of retinene. He introduced the term "cephalopsin" for the photostable pigment of squid retinas and noted that, although its absorption spectrum was like that of rhodopsin in vertebrate eyes, it differed chemically. St. George and Wald (1949) repeated this work with somewhat different technique and obtained a bleachable pigment that showed no important distinction from vertebrate rhodopsin.

Other approaches to invertebrate photochemistry have been mostly measures of spectral sensitivity in the intact animal.

### PHOTOSENSORY STRUCTURES

From the anatomical side, numerous attempts have been made to keep the record up to date (Carrière, 1885; Beer, 1901; Hesse, 1902; Parker, 1922; Plate, 1924; Uexkuell and Brock, 1927; Hess, 1943; Kahmann, 1947; and the long series of short papers by Hilton, 1920-1941). Most of these provide brief treatments of the gross histology of sense organs or receptor cells and indicate the limitations inherent in each of the several levels of organization. In general, however, only articles with a more limited scope provide much information about the relation between structure and function, presenting the subject in terms of the reacting animal.

In the following account the terminology applied to each photosensory organ is intended to indicate something of its structure. In protozoans having a specialized photoreceptor within the single cell, it is termed a *stigma*. In multicellular animals the photoreceptor may be a specialized neuron of the nervous system but may lack conspicuous pigments; this is described as a *neuronal photoreceptor*. If the photosensory structure is unicellular but has obvious pigment associated with it, giving its sensitivity a measure of directionality, the word *eyespot* is applied. Multicellular photoreceptors, by contrast, are called *eyes*. They may be *ocelli*, or "simple eyes," if a number of receptor cells are grouped into a retina consisting of more than a single circle; light may reach the retina through a pinhole in a pigment diaphragm (as in *Nautilus*), through the cavity of an open, hollow cup (as in various gastropods), or through a single lens. Or the eyes may be *compound eyes*, composed of individual *ommatidia*, in which the receptor cells form a single circle like the segments of an orange and light reaches them through a lens system along the axis of symmetry of the group.

Compound eyespots are known in some annelids and some mollusks.

Compound ocelli occur in a few arthropods and echinoderms. Solitary ommatidia, or structures so similar anatomically (though perhaps not homologous) as to be identified as such, are characteristic of some insects.

Collectively the receptor cells of a multicellular eye may be regarded as the retina, and each cell as a retinula. Often a retinula is specialized into a nucleus-containing basal portion and a distal segment containing radiating fibrils termed *sensillae*. The grouped receptor cells in an ommatidium often secrete along their common boundary a refractive body, the *rhabdom*, which extends the optical axis to the most basal part of the retinulae and supposedly transmits to the photosensory cells light received through the more distal dioptric system.

#### PROTOZOA

Where a definite pigment spot is present in a protozoan, the photosensory mechanism has seemed more evident, but amebas and ciliates lacking such pigmented specializations react also to light. Hertel (1904), investigating the effect of ultraviolet at  $280\text{ m}\mu$ , concluded that the responses to irradiation arose from catalysis of hydrogen peroxide formation. Harrington and Leaming (1899), using the spectral regions visible to man, noted a differential effect with wave length such that amebas traveled rapidly in radiation of long wave lengths (red), but protoplasmic streaming was retarded, stopped, or reversed by rays toward the violet end of the spectrum; white light seemed even more effective in affecting activity. Mast (1932) and Mast and Stahler (1937) concluded that increased illumination increased the elastic strength of plasmagel and inhibited pseudopod formation, that there was no threshold for response, and that the "all-or-none law" did not apply. The rate of locomotion, however, was dependent upon both the intensity of illumination and the state of light adaptation of the ameba, with an optimum reached at about 15,000 m-c after an exposure of some minutes. Decrease in rate of movement with further increase in light intensity was believed due to the "gelating action of the shorter waves of light."

Some doubt was thrown on the adequacy of these explanations by the work of Folger (1927), in which the effect of sudden illumination in inducing cessation of activity in amebas was found to be interchangeable with the effect of mechanical shock; both showed a definite reaction time, a latent period, and a refractory period. Mechanical shock affected the reaction to light, except that, when an ameba formed a food cup, it failed to respond to light stimulation, and after the cup was formed, the stimulation period and reaction time were sometimes shorter than usual. After a response to light the ameba must recover before it will respond to shock. Hence the two stimuli must act in the same way, and since temperature has little, if any, effect on reaction time, the mechanism is quite obscure.

The work of Schaeffer (1914, 1917) makes understanding of the light

sensitivity of amebas even more difficult. He found that amebas detect vertical light beams  $20\ \mu$  in diameter at a distance of  $100\text{--}150\ \mu$  (perhaps from scattered light), usually move toward the beam, do not react if it is extinguished before they reach it, and may proceed through the beam or react negatively on entering it, with no obvious difference in the effect of white or spectral colors. Food (globulin grains) laid over a beam of intense white or blue light was "sought" and ingested, but over green or dark pencils in a general field of vertical illumination it was frequently avoided. Dark pencils in the light field were detected at a distance, as were light pencils in a dark field, though scattering could scarcely be invoked as an explanation for the former and no mechanism allowing this was found. In a 1929 paper Schaeffer reported that, when amebas moving around glass rods are stimulated by light, they significantly alter the ratio of right turns to left turns. He postulated two kinds of protoplasmic constituents, similar to isomers, a right-turning and a left-turning, which are produced or destroyed at different rates depending on the species of ameba and rate of growth, are stable for hours under ordinary conditions, determine the ratio of right turns to left turns in locomotion, and are affected differentially by light on a temporary basis in so far as their power to determine the direction of the ameba's apparently random path is concerned. This too seems a far cry from conventional photochemistry.

The orientation and free movements of *Paramecium* and *Stentor* subjected to sudden changes in illumination have been studied without reaching much understanding of the photosensory mechanisms. Most responses found can be laid to "general irritability" rather than to specific reactions to light or directional effects. Fox (1925) reported upward swimming by *Paramecium* in darkness, downward in light, with the short wave lengths most effective in altering the direction.

Among flagellates, definite pigment organelles ("stigmata") are known in many forms. Mast (1928) summarized previous work with the generalization that in unicellular flagellates the stigmata consisted of a "spoon-shaped pigmented structure and a hyaline mass which contains photosensitive substance." Furthermore these appear to be connected directly or indirectly to the flagellar mechanism. Because of its position the pigment mass may cast a shadow in directed light over the "hyaline mass," stimulating greater or lesser activity toward the light, so that the sum of apparently random movements shifts the organisms toward or away from a light source (Fig. 14-3). This is the situation in *Euglena*, *Trachelomonas*, and some others.

In *Chlamydomonas*, however, Mast found (1916, 1928) that the pigmented region is elliptical and dishlike in being concave, with a maximum diameter of over  $2.5\ \mu$  in some specimens. This stigma is located at the surface of the body under a slightly projecting hyaline biconvex lens

fitting the concavity of the pigment and concentrating light. Presumably the pigmented structure contains photosensitive materials, since the organism responds to light—orienting fairly precisely in directed beams—with a maximum sensitivity in the green near  $500\text{ m}\mu$ .

The stigmata of *Volvox*, *Gonium*, and other chlorophyll-bearing colonial protozoans consist of a cup-shaped pigment mass, with a lens at the mouth of the cup and the light-sensitive material located between the lens and the bottom of the cup. Again the photoreceptive substance appears connected with the flagella by means of a differentiated neuro-motor or conducting system. The lens is inferred from the fact that

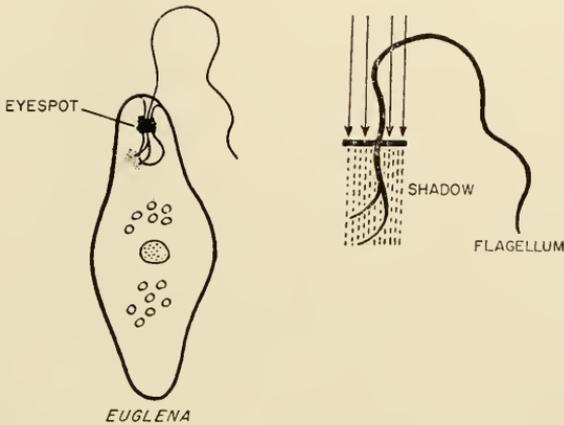


FIG. 14-3. A pigment mass within an organism may cast a shadow. This seems to be the basis of directional movements in some unicellular forms. If flagellum activity is inhibited by light on the bases and full action follows shading there, then average (random) movements become directional toward a source because of the pigment spot. (After Wager, 1900.)

incident directed light is concentrated into two foci—a yellowish one in the wall of the pigment cup near its outer surface, and a bluish-green one in the cup near the inner surface. The walls of the cup transmit longer light waves (red to yellow) but reflect the shorter waves to the second focal point. Most believed the concentration of longer waves to be incidental. But since each zooid in the colony has one such stigma, the orientation of the pigment cup is definitely related to the structure of the colony in such a way that lateral illumination (in terms of the axis of symmetry) produces alternate shading and exposure of the lens mechanism in each stigma as the colony rotates on its longitudinal axis. Illumination from directly in front or behind furnishes no concentration of light behind the lenses. In general, zooids toward the anterior pole of the colony in *Volvox* and *Gonium* possess larger stigmata—as much as  $3\ \mu$  in diameter—but zooids at the opposite pole contain similar structures many times smaller. Mast concluded that the stigmata served as direction eyes

and that light focused toward the periphery of each pigment cup was only one-ninth as effective as that reaching the center of the sensory area.

In flagellates without stigmata, such as *Peranema*, Shettles (1937) and Shortess (1942) concluded that the whole cell was photosensitive in a gradient from least in the posterior end to most in the flagellum. Even detached flagella responded to increased illumination by bending, although no recovery seemed possible except in intact specimens. A reasonable spectral-sensitivity curve was obtained from these studies (Shettles, 1937); dark and light adaptation indicated definite change in the photosensitive substance.

#### COELENTERATA

The earliest comments on the light relations of invertebrates concerned *Hydra* (Trembley, 1744). In this animal, with no visible photoreceptors, Wilson (1891) reported movements into parts of an aquarium shaded with blue glass; he claimed that this was independent of intensity for both brown and green *Hydra* exposed to several colors simultaneously. Haug (1933) found no correlation with wave length, but a shadow reaction seemed definite at the oral end. Both Haug (1933) and Schluensen (1935) investigated the ability of *Hydra* to distinguish between light directed toward it from various directions, singly or in multiples. Lack of a spectral characteristic seems peculiar, however, since the mechanism might be expected to be similar to that investigated in the hydroid *Eudendrium* by Loeb and Ewald (1914) and Loeb and Wasteney (1917), in which good correlation was found between prediction based on the Bunsen-Roscoe product law of intensity and exposure time and activity observed in orientation of the polyps to directed illumination.

Driesch (1890) reported on the accuracy with which the hydroid *Sertularella* was oriented with respect to lateral illumination. Bohn (1906) suggested that the anemone *Actinoloba* reached a position with the oral pole toward the light by a series of trial-and-error movements. Moore (1924, 1927), however, found remarkably precise orientation in *Cerianthus*, with the angle turned proportional to the logarithm of intensity, and a spectral sensitivity having a maximum between 510 and 570  $\mu$ . McClendon (1910), Schmid (1911), and Gee (1913) with other anemones described the tentacles as expanded in light, retracted in dark. McClendon explained the activities and position of *Cradactis* in rock crevices as due to a negative response to light in the foot combined with a positive response in the tentacles. Gee offered the hypothesis that in *Cribrina* the light reaction in the tentacles was due to photosynthetic products from symbiotic algae in this anemone. Since the phenomenon is widespread and the symbionts are not, general sensitivity without organized photoreceptors seems a more adequate explanation.

Ocelli associated with the tentacles of medusae have been illustrated

by many anatomists. Yerkes (1902, 1903, 1906), Morse (1906, 1907), and Murbach (1907, 1909) studied the light reactions and structure of *Gonionemus*. Murbach (1907) concluded that the light reactions showed the marginal sense organs to be only part of the photosensitive mechanism. Light appeared necessary for upward swimming but acted as a general photokinetic stimulus, with gravity providing orientation and direction.

#### CTENOPHORA

Photosensitivity has been demonstrated only indirectly in comb jellies. Heyman and Moore (1925) and Moore (1926) approached light sensitivity of *Beroë* and *Mnemiopsis*, respectively, in terms of inhibition of luminescence and found clear indication of dark adaptation.

#### PLATYHELMINTHES

The conspicuous ocelli of *Planaria* and other free-living turbellarians have been described in detail. In all the organ consists of one or more receptor cells surrounded by a cup-shaped group of pigment cells. In *Prohynchus* the ocellus is at its simplest, with a single pigment cell cupped around a single photosensitive cell, the latter composed of an inner rhabdom with the nerve-fiber endings, a middle ellipsoidal lens, and an outer transparent "myoid" section containing the nucleus. Kepner and Foshee (1917) noted considerable change in the gross form of this ocellus as it became dark- or light-adapted. Hesse (1897) investigated the angular coverage of the ocellus in *Planaria*. Other workers experimented with removal of the eyes unilaterally or bilaterally and found that photokinesis continued without eyespots but that directionality was lost. Unilateral "blinding" resulted in loss of directional responses from that side but no circus movements. Dermal photosensitivity (structural basis unknown) seemed responsible for spectral sensitivity. Beuther (1926) claimed a difference in response to red and to yellow both over Hering's colored papers and to filtered light beams; Erhardt's repetition (1932) and extension of this work explained all reactions on the basis of brightness discrimination. Werner (1926) noted that a considerable part of the sensitivity to ultraviolet was as a dorsal dermal photosensitivity. He believed that responses were to energy in ultraviolet wave lengths directly, not to secondary fluorescence in the visible spectrum. Merker and Gilbert (1932a,b) found strong responses to ultraviolet (366–313 m $\mu$ ) but much less response to fluorescence; the planarians exhibited orientation, photokinesis along the resultant of two opposed beams of ultraviolet, and response to ultraviolet alone when between opposed beams, one of which was ultraviolet and the other a fluorescence-eliciting wave length. Response to ultraviolet was pronounced also in contrast with an incandescent-lamp beam. Merker and

Gilbert, however, reported that headless planarians were highly kinetic under ultraviolet stimulation and that their movements lacked directional qualities, but that one ocellus allowed orientation and avoidance; thus they were able to plot the visual fields of *Planaria* toward ultraviolet. Further experiments with multiple lights and color have contributed little new to an understanding of the photoreceptors involved.

Taliaferro's findings (1920) indicated that the point where the planarian body bent in responding to illumination of the ocelli depended on the

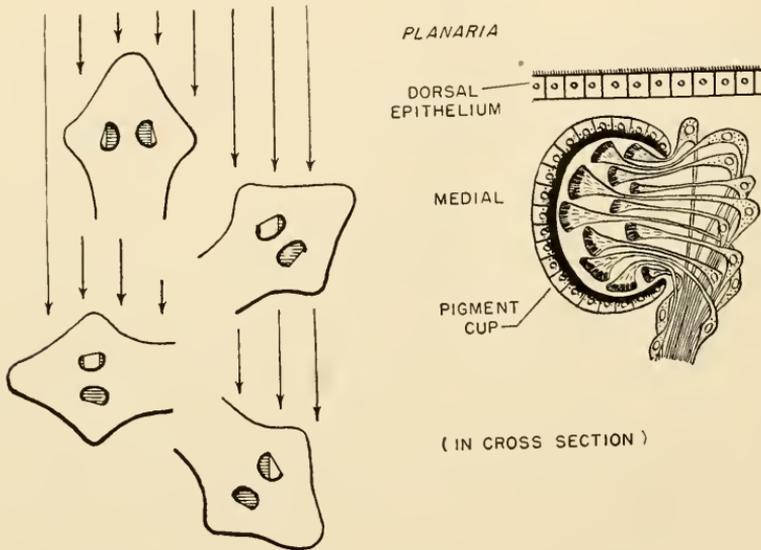


FIG. 14-4. A pigment cup limits the light rays that can enter, but the resolving power of the system is increased materially if the sensory cells show a diverging pattern and greatest sensitivity when illuminated along their axes. (After Hesse, 1897.)

intensity—the higher the intensity, the more posterior the bending, although the bend always occurred in front of the pharynx. Furthermore the sensory cells in the posterior and ventral portions of the pigment cup were involved with responses turning the animal toward the eye of that side, whereas illumination of the other receptor cells was followed by a turn in the opposite direction. Removal of part of the ocellus provided complex modifications in the response, since the anterior portion includes the nervous connections to the central integrating center. Each receptor cell, moreover, responded only in proportion to the amount of light traveling along its longitudinal axis. Hence the pigment cup provides a degree of shading for the entire mechanism, but the directional response is much more precise because of polarity of the receptor cells contained within it (Fig. 14-4).

Eyespots are found in the parasitic trematodes—in the monogenetic *Polystomum*, in various cercariae, in the miracidium of *Fasciola*, and in

various stages in the life history of digenetic forms. Faust (1918) concluded that these findings demonstrated the fundamental significance of eyespots, that the "preservation of the pigmentation and of the 'lens' in this or that group has been decidedly erratic and apparently unrelated to the systematology of the group," that the major trend was toward degeneration, and that pigmentless eyespots no doubt exist in many species. Three cases of this were reported (*Cercaria racemosa*, *C. gracilima*, and *C. minor*); although they are undoubtedly functionless, the existence of the unpigmented structures "extends the knowledge of the extent of eyespots and of the types of degeneration that have occurred under conditions of endoparasitism."

#### NEMERTINEA

Hilton (1921a) reported that photoreceptors were usually present along the sides of the head—sometimes a single pair, more often in one or more groups on each side. In the simplest condition these are mere eyespots, but more complex ocelli have a limiting membrane around a fluid supported by strands from nearby cells. This mechanism serves to focus light on sensory cells of somewhat rodlike form, enveloped in pigment except toward the fluid sphere and connected to the cephalic ganglion by nerve fibers. Minkiewicz (1906a,b) indicated strongly negative light responses in *Lineus*, one of this group of worms.

#### ASCHELMINTHES

Among rotifers some genera possess no organized photoreceptors, but in most there are at least two eyespots that, in their simplest form, consist of a refractive globule in a red pigment cup to which nerve fibers pass. In some these supposedly visual organs rest directly on the cerebral ganglion as "cerebral" eyespots; in others they are "apical" in position, situated on the rostrum; in a few the corona (wheel organ) bears lateral eyespots, and these may be combined with true ocelli in the other locations. Viaud (1938a,b,c, 1940, 1943) has been able to demonstrate that rotifers reacting to light do so both through a general dermal photosensitivity present even in eyeless forms (as shown by speed of locomotion) and, where eyespots or ocelli are present, under the directing effect of these specialized organs. The two photosensitive systems, moreover, show unlike spectral-sensitivity features that indicate either the effectiveness of the red pigment or an actual dissimilarity in the photosensitive basis of response.

Among the nematodes, responses to light have been reported by Wuelker (1924) without comment as to the mechanism involved. Hertel (1904) included nematodes in his study of the 280-m $\mu$  ultraviolet line from the magnesium spectrum and concluded that catalytic formation of hydrogen peroxide explained any responses shown.

## CHAETOGNATHA

*Sagitta* and its relatives have two ocelli located dorsolaterally on the head. According to Hilton (1921b), each is globular, with three biconvex lenses separated by pigment; the lenses concentrate light on groups of rodlike sensory cells. Russell (1931) looked into variations in the vertical distribution of *Sagitta* in relation to light at various depths of the sea but reached no specific conclusions regarding the photoreceptors.

## ANNELIDA

Hilton again (1924) has provided the chief summary of anatomical information regarding the eyes of archannelids. In *Dinophilus* the eyes consist of two semicircular pigment spots in the head region; in *Nerilla* there are four eyespots, the anterior pair more elongate and directed outward and forward, the posterior pair pointing outward and backward. Nothing is known of their physiology.

*Polychaeta*. The polychaetes, however, afford far greater range in photoreceptors. Both Schreiner (1898) and Hesse (1899) attempted to trace a phylogenetic sequence through progressively more complex forms, from epithelial eyespots without separate lenses, through ocelli with lenses as in *Nereis*, to the highly complex, camera-like eyes of *Alciopa* and *Eupolyodontes*. Unfortunately the anatomical information is not matched by studies of the response characteristics of the various mechanisms, so that it would appear that many with highly specialized eyes achieve no more complex shadow reactions or true vision than those depending on dermal photosensitivity or simple pigmented eyespots. Thus in *Potamilla*, where there are numerous modified epithelial cells in patches on the main stems of the cephalic branchiae, each is composed of elongated pigment-bearing cells, a few of which possess in their distal ends refractive bodies that seem to concentrate the light internally on a supposedly photosensory portion of each cell. Every sensory element is separated from its neighbors by pigment cells, making it a true compound eyespot. Similar structures are found in many sabellids and serpulids (such as *Branchiomma*, Fig. 14-5), yet the known reactions of these forms seem not to differ from others having far simpler morphological details.

The ocelli of *Nereis* (Fig. 14-6), *Potamilla*, and the like lie under lenticular thickenings of the cuticle, but the eyes themselves are composed of a single layer of epithelium. A series can be arranged showing progressive enlargement and infolding of the cuticular lens until it forms a conspicuous spheroidal body around which the sensory cells are arranged almost radially, and the whole is enclosed by small pigment cells except for an outer zone through which light enters (Fig. 14-6). In *Branchiomma* and some others the sensory layer around the cuticular lens is double, with the pigmentless sensory cells separated from the lens by

hyaline cells. These latter contribute a "vitreous body" separating the photosensitive mechanism from the lens. Brunotte (1888) described the anatomical details of these compound eyes in *Branchiomma*. Von Buddenbrock (1934) examined the behavior of the worm without finding anything spectacular—it became photokinetic when facing a black screen

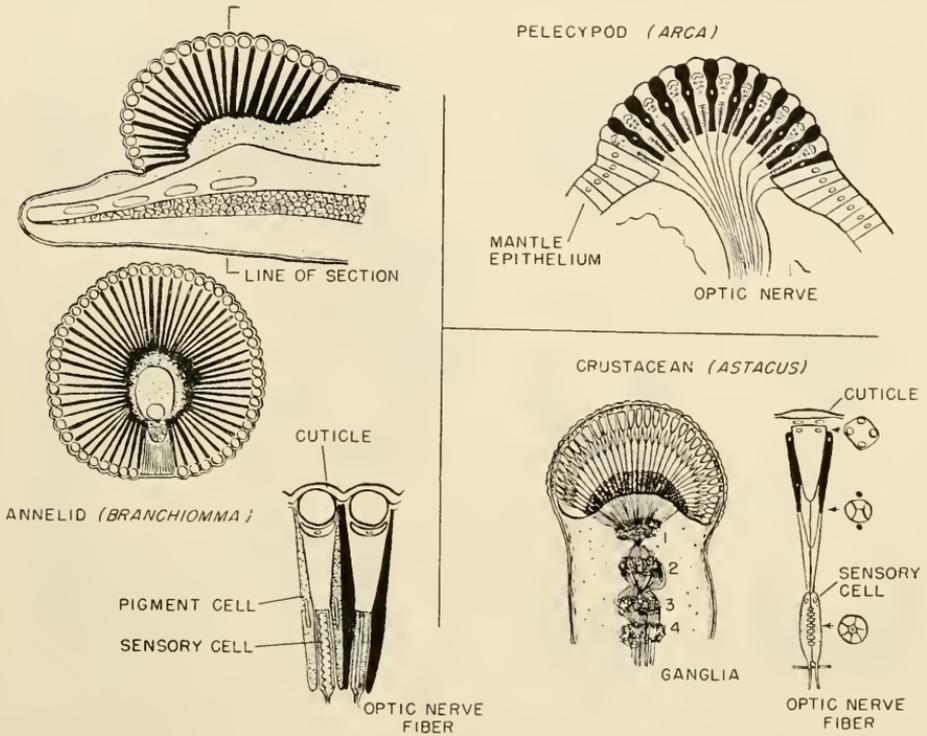


FIG. 14-5. Grouping of visual units is found in annelids, mollusks, and arthropods, but is most familiar in the last-mentioned phylum and is most diversified and specialized there. In the annelid *Branchiomma* each unit includes several sensory cells, closely analogous in arrangement to those of such crustaceans as *Astacus*; both of these groupings are hence compound eyes composed of ommatidia. In the mollusk *Arca*, by contrast, the units are unicellular eyespots, and the grouping is a compound eyespot. Sections are shown at three different levels through an ommatidium of *Astacus*. (*Annelid after Hesse, 1899; pelecypod after Kuepfer, 1915; crustacean after Giesbrecht, 1921.*)

or facing away from a white screen but otherwise showed no reaction! The camera-like alciopid eye, with its muscles allowing accommodation by shifting the sensory tissue with respect to the dioptric apparatus, has been described by Greeff (1875, 1877) and others, but again the function is not known to attain the limits allowed by its organization. The accommodation feature was investigated by Hess (1918) and Demoll (1909a). A similar eye in *Eupolyodontes*, according to Pflugfelder (1932), is positioned in such a way as to be usable for binocular vision and depth

perception. Careful investigation of how far these worms use their remarkable visual apparatus is still to come.

*Nereis*, *Eunice* (= *Leodice*), and others with known habits of swarming in definite relation to light have been examined in terms of visual organs—*Nereis* by Andrews (1892), Langdon (1900), and Mosella (1927) and *Eunice* by Schroeder (1905) and Hess (1931). Mosella reported a

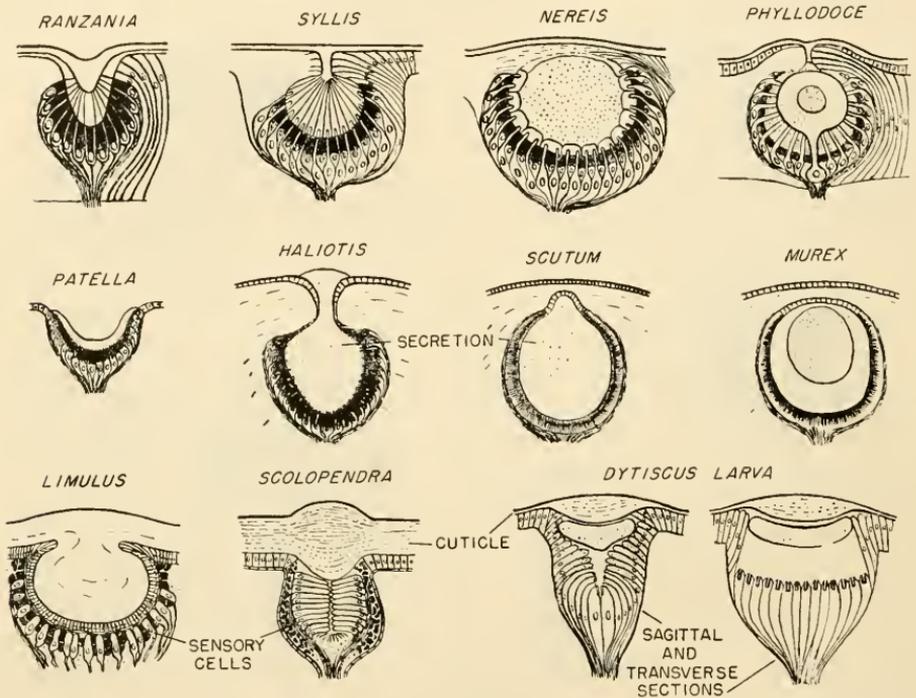


FIG. 14-6. Analogous series of photoreceptor specializations can be traced in annelids (top), mollusks (middle), and arthropods (bottom). From a simple cup lined with sensory cells, various provisions for lens action are found. Separation of the sensory layer from the lens may permit formation of an image and potential increase in resolving power. (*Limulus* after Demoll, 1914; *Scolopendra* after Heymons, 1901; *Dytiscus* after Guenther, 1912; others after Hesse, 1899 and 1901.)

structural asymmetry of the photosensory epithelium which would adapt the anterior pair of *Nereis* ocelli to lateral and forward vision and the posterior ocelli to upward vision. Response to light has been investigated in *Nereis*. The effect of each ocellus was checked, and alterations in responses were studied when nerve connections were severed in various combinations. Herter (1926) concluded that the direction of movement of the animal had no relation to the direction of illumination, but that the anterior ocelli mediated negative responses to light, and the posterior ocelli, positive responses. Brand (1933) found that, with any one ocellus intact on either side, the animal behaved as though unilaterally blinded and that removal of half the supra-esophageal ganglion without destruc-

tion of the ocelli gave the same unilateral blinding effect. Hess (1931) concluded that the reactions to light intensity were mediated through dermal photoreceptors along the middorsal line, sides, and anterior and posterior ends of *Eunice*, but that the ocelli served visual functions apart from those eliciting photokinetic responses. Other anatomical studies on polychaetes involve the compound eyespots of *Spinther* and the receptors of *Polygordius*, *Tomopteris*, and *Scolecoplepis*. Behavior studies in

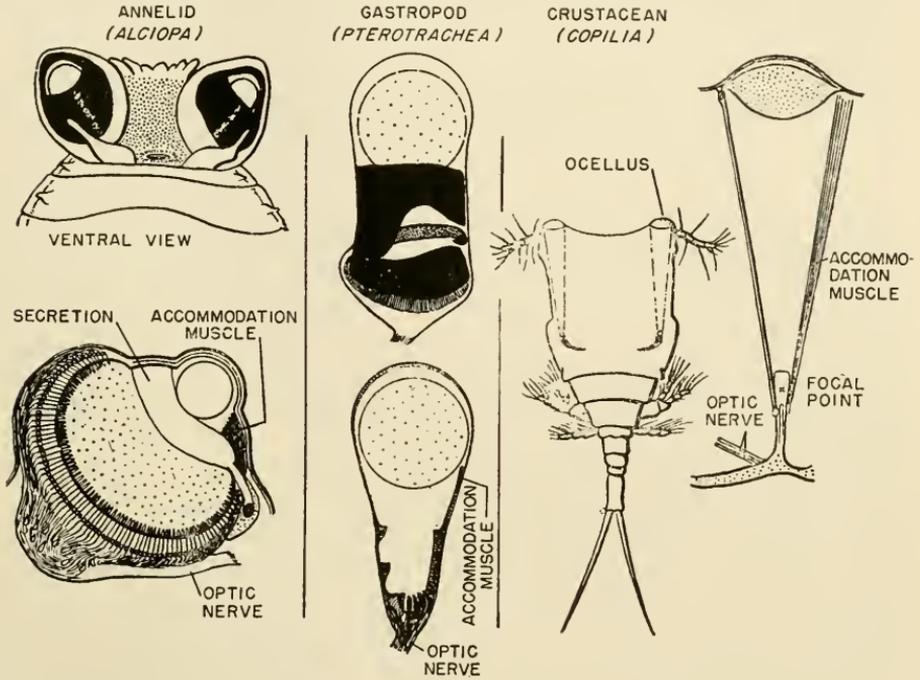


FIG. 14-7. The camera-style eye is represented not only in the cephalopod mollusks but also among the annelid worms and, with specializations, among the gastropod mollusks and plankton crustaceans. The *Alciopa* eye appears to provide a good image of fair size, but the sensory mechanisms in *Pterotrachea* and *Copilia* must function more as sights for aligning the body with reference to light. All three provide for accommodation. (*Annelid Alciopa* after Demoll, 1909b; *gastropod Pterotrachea* after Hesse, 1910; *crustacean Copilia* in dorsal view after Giesbrecht, 1890; detail after Grenacher, 1879.)

terms of photoreception include work on the serpulid *Hydroides*, on *Sabellaria* larvae, and *Clitellio*.

*Oligochaeta*. Possibly the wider availability of earthworms has made easier the study of this more degenerate type of organization. Neuronal photoreceptors in the skin have been recognized and described by Langdon (1895), Hesse (1896), and Hess (1925). Reactions to light in these animals (*Allolobophora*, *Eisenia*, *Lumbricus*, *Perichaeta*, and *Pheretima*) have been analyzed. Extension of the body exposes more photoreceptors and hence increases the worm's sensitivity to light (Harper, 1905); in

weak illumination this gives rise to complex behaviors, but in stronger light orientation is much more accurate. The supra-esophageal ganglion is not necessary for reactions to light or for photic orientation (Hess, 1921, 1924, 1925), but with ganglion and anterior end intact, light sensitivity is higher and responses different in direction (positive to light without ganglion or anterior end of body, negative to light when these are intact). Segall (1933) also found reactions following intensity regardless of area exposed by any given degree of extension, and hence no central summation in the nervous system. Unteutsch (1937), on the other hand, reported a definite relation between reaction time and area of worm exposed to light, with increase in time following increased percentage of total surface illuminated. This worker stated further that the maximum sensitivity to shadowing is in the yellow region of the spectrum, whereas that for general light perception is in the blue; sensitivity to shadowing was distributed uniformly, and that to illumination was greatest at the two ends, with a maximum at the anterior end and a minimum at three-quarters of the length toward the anus. From these data two separate receptor systems were postulated, with correlation between light and shadow effects at a segmental ganglionic level rather than being mediated centrally. No anatomical basis for these two types of receptor has been reported.

*Hirudinea*. Leech ocelli have been described extensively. They appear to be the chief specialized sensory organs and in the first five body segments appear to replace the lateral line organs. Each ocellus is approximately cylindrical, with its longitudinal axis at right angles to the body surface. An outer layer of dark pigment surrounds a number of clear refractive cells. The nerve fibers enter the organ at one side and continue up the axis to the several sensory cells. Whitman (1893) concluded that they were modified tactile elements; Meryll (1894) distinguished between the photosensory structures and those which were associated and served the sense of touch. Apáthy (1902) distinguished three phylogenetic steps in their specialization. Even the pattern of eye arrangement is characteristic of the various genera. Behavior aspects have been described by Bohn (1907), Herter (1929), and Schlueter (1933). Response to light depends on the state of nutrition and involves both the eyes and general dermal photosensitivity. Schlueter severed the nerve connections to the five eyes on one side in *Hirudo* but produced no asymmetry of response. Again the light reactions appeared not to be mediated through the central nervous system as an image of the outside world. Yet reactions to light intensity in each eye have been reported, in terms of altered pigment distribution, in work by Stschegolew (1927) and Wells (1932). These seeming discrepancies between behavior and structural possibilities emphasize a point made by Parker (1922), i.e., that the presence of photoreceptors does not mean that an animal can

see. In many animals, as in jellyfishes, nerves from sense organs pass directly to muscles or via ganglia to muscles. Hence activities due to light or shadow correspond more closely to those of our own pupil control. Higher development of nervous centers in relation to visual organs allows a second function, informing the animal about its surroundings. This is distinct from and superimposed upon the delivery of impulses along nerve fibers that excite muscles to action. These are the light aspects of the neurological statement made by Loeb (1894), when he pointed out that there is no apparent parallelism between brain function in any species of worm and its systematic position and that the independence of parts of the body and of physiological processes (including photosensitivity) varies from one to another without clear relation to phylogenetic bases.

#### BRYOZOA

Response to light by *Pectinatella* larvae and by *Lophopus* was reported by Marcus (1925), but no special sensory mechanisms have been studied.

#### MOLLUSCA

*Amphineura*. The discovery of eyes here began with two papers by Moseley (1884, 1885) reporting these structures on the outer surfaces of the shells on exposed areas. Mostly these ocelli were oval or circular in outline, from 65.0 by 42.5  $\mu$  in *Corephium* to 0.73 mm in diameter in *Acanthopleura*. Each has a highly refractive corneal lens surrounded by a narrow zone of dark pigment, but both the presence of ocelli and the manner of their arrangement are characteristic of the various genera of chitons. Plate (1897, 1899) provided information about these structures and distinguished clearly between the ocelli and two types of "esthetes"—macroesthetes, where several sensory cells were clustered below a cuticular thickening, and microesthetes, which are unicellular. Heath (1904) traced ocellus formation in several genera and believed it improbable that the ocelli were functional only in larval stages. Thus an adult *Corephium* may have as many as 3000 ocelli on the anterior plate of the shell, with perhaps 8500 as a total number for the whole organism. Behavior studies, however, place no emphasis on these supposedly photosensory structures. Crozier and Arey (1918), Arey and Crozier (1919), and Crozier (1920a) found a gradual change in response to light, from negative to positive, as *Chiton* aged and grew larger. Areas bearing ocelli were sensitive, but so too were the whole dorsal surface of the scaly girdle and even the soft ventral surface of the foot. Sudden increase in illumination of the ocelli failed to elicit responses, although similar treatment of the girdle was effective. The whole dorsal surface was sensitive to shading, and the activity of the animal depended on the steady illumination received there. Shadow reactions were obtained most strongly

by stimulating the periphery of the girdle. Dark adaptation at night, with increased sensitivity, was reported.

*Pelecypoda*. In the pelecypods a very considerable range of photo-sensory structures is found. Some, like the scallop *Pecten*, possess conspicuous eyes. Others, such as the clams *Venus* and *Mya*, are often referred to as eyeless. Nagel distinguished between "skioptic" forms such as *Venus*, *Ostrea*, and others sensitive only to shadowing; "photoptic" forms such as *Lima* and *Mya* which are sensitive also to brightness level; "photoskiotics" such as *Pholas* which respond to both increase and decrease in intensity; and "ikonoptics" such as *Pecten* in which the structure supposedly allows image formation. Sharp (1883) reported specialized pigment cells between the bases of the tentacles of the external edge of the siphon in *Solen* and suggested that the shadow reaction was due to their operation. Dubois (1889a,b) investigated reactions in a similar system for *Pholas*. Corresponding mechanisms are known in *Mya* (Hecht, 1921a,b,c; Light, 1930; Koller and von Studnitz, 1934) from both structural and physiological viewpoints.

A rudimentary pigment cup, sometimes with a lens, has been described in *Mytilus*. In some species of *Lima* the mantle eyes consist of a cup-shaped columnar epithelium of alternating retinula cells and pigment cells; the latter secrete a vitreous layer enclosing the inner knobbed ends of the retinulae, and the optic nerve fibers collect together outside the cup (Kuepfer, 1915). In *Potamides*, by contrast (Pflugfelder, 1930), there is a distinct lens, a vitreous body surrounded by a single layer of retinulae, but the nerve fibers emerge on the illuminated side of the retina, so that it is inverted in the vertebrate sense. In *Cardium* also, where the mantle bears many eyes, each has a thin cornea, a large multicellular lens ovoid in form with its long axis parallel to the optic axis, and a columnar retina separated from the lens by the optic nerve fibers passing toward the optic nerve. The retinal cells are inverted also in *Pecten* (Fig. 14-8), but there the retina is unusual in being double, with both levels including photoreceptors directed away from the lens. Hartline (1938) studied electrical impulses in the optic nerve of *Pecten* when the eye was illuminated and found that the proximal receptor layer responded to steady illumination and that the distal layer generated an off-discharge forming the presumptive basis of the animal's strong shadow reaction.

The lens in *Pecten* is a much more nearly biconvex body, separated from the retina by enough distance to allow image formation. Wenrich (1916) investigated this and found that the smallest white card, movement of which produced a response in the scallop, was a 15-mm square at 35-cm distance, indicating an acuity of  $2^{\circ} 28'$ . An alternative and more probable explanation of the observed fact is that, at the light intensity used, the appearance or disappearance of the card in the visual field

furnished the minimum effective change in intensity. A smaller card at higher intensity or a larger card at lower intensity would probably have been equally effective. Detailed anatomical knowledge of the eyes of various species of *Pecten* is based on a series of papers from that by Grube (1840) to those of Kuepfer (1915, 1916). A similar mechanism is present in *Spondylus*.

A very different visual structure is found in the attached *Arca*, particularly *A. noae*, and in the free-swimming *Pectunculus*. Thus Patten

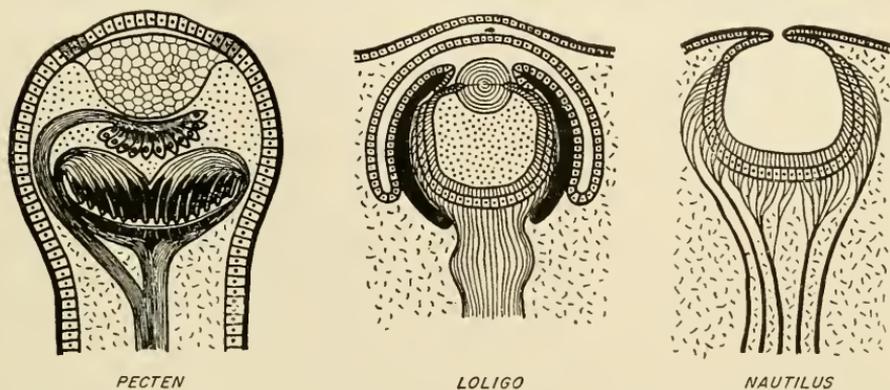


FIG. 14-8. Mollusk eyes provide for varying degrees of image formation. In the scallop *Pecten*, the two levels of sensory cells differ in function: those nearer the lens mediate only an off-response, whereas those farther from the lens respond to steady illumination. A reflecting tapetum (shown as dark blocks basal to the tips of the receptor cells) increases sensitivity to low-intensity stimulation. In the squid *Loligo*, reflex adjustments of a pupil, accommodation, and gross movements of the eye through contraction of extrinsic musculature all provide a close analogy to the vertebrate eye. The pinhole camera eye of *Nautilus* becomes functional and definitive at a stage which is passed through in the embryonic stages of other cephalopod eyes. (Pecten after Kuepfer, 1915; *Loligo* and *Nautilus* after Hensen, 1865.)

(1886) recorded *A. noae* 8.5 cm long with 235 separate eyespots projecting from the mantle margin. Each eyespot is either single or compounded of as many as 70 per compound eyespot (Fig. 14-5). Each unit in a compound eyespot is isolated from its neighbors by pigment cells extending well up between the externally convex corneal-lens surfaces. Optic nerve fibers form a brushlike sensory net in the base of the receptor cells, so that each is responsible for a sector of surrounding environment in a manner analogous to the compounded ommatidia of crustaceans and insects.

"Eyes" described by Vaillant (1865) in the protruding mantle of the giant clam *Tridacna* were referred to by Brock (1888) as glandular rather than visual, but Yonge (1936) upholds the light-concentrating function of these protruding hyaline bodies. Instead of a sensory function, however, these curious structures diffuse light within the mantle and permit more rapid growth and reproduction of the algae embedded in the mantle

tissue—algae that these clams engulf as food through the action of amebocytes as a substitute for filter feeding. Thus the hyaline bodies are not photoreceptors but are part of the nutritional system.

*Gastropoda.* Among the gastropods a considerable range of photo-sensory structures is known. The limpets *Patella* and *Acmaea* have a simple pit lined with retinula cells that secrete a thin hyaline covering conforming to the inner contours of the pit (Fig. 14-6). The abalone *Haliotis* has a far deeper pit, and the secretion not only fills it but expands into a convex light-concentrating structure on the body surface. Key-hole limpets such as *Fissurella* have a closed sac just below the surface, with no secretion—hence an “outer” and an “inner” cornea. In many other snails the ocellus is like that of the whelk *Murex* in having a large lens eccentrically placed in the secretion filling the optic space, so that the retina is somewhat remote from the lens, and an image of sorts may be formed. Land snails such as *Helix* frequently have such large lenses that the secreted hyaline spacing is reduced to a thin film and no satisfactory image seems possible. These comparative aspects (Fig. 14-6) have been furnished by various workers. Willem (1891, 1892) concluded that the tactile sense of terrestrial forms was so well developed that they paid little attention to light and could distinguish form only at 1–2 mm and voluminous objects at no more than 1 cm. He further summarized his findings by indicating that aquatic pulmonates have no distinct vision at any distance and that all the pulmonates chiefly depended on dermal photosensitivity. Yung (1913) claimed that *Helix* eyes were blind, because he could demonstrate a lack of continuity in the optic nerve—a gap in it between eye and cephalic ganglion. Others have extended the generalization regarding dermal photosensitivity to various slugs (*Arion* and *Limax*) and snails. Yet physiological investigations of these same animals indicate circus movements when one ocellus is stimulated, as in nondirectional light with one eyestalk tip (and ocellus) removed. Crozier and Libby (1925) reported a temporary loss of light responses in *Limax* after meals of cooked potato but not after raw-potato ingestion. Mitsukuri (1901) found that *Littorina* responded to light in a different sense depending on whether the animal was wet or dry. Bohn (1904a,b, 1905a,b) investigated these reversals of response; Dimon (1905) extended the same principle to the mud snail *Nassa*. Fraenkel (1927) noted that, when *Littorina* were inverted, they responded positively to light; when upright, negatively. The complex alterations of response are believed to explain migrations of the animals with respect to land and tidal movements.

A far higher degree of visual response was seen in the opisthobranch *Elysia*, where a visual field could be investigated for the pigment cup at the base of the tentacle (*ibid.*). Dijkgraaf (1935) measured a latency of 0.5 sec in the contraction of the marine snail *Aplysia* stimulated by

light. Smith (1906) found pigment migration in the retina of *Planorbis* but uncovered no clue as to its causation. In the nudibranch *Chromodoris* the small eyes situated beneath the skin were believed an important part of the receptive mechanism for general photic response, but the gill crown responded to shading independently, apparently as a result of sensory structures in the branchial collar. Even a single genus may show great variation. Thus the species of *Onchidium* (a pulmonate gastropod) examined by Crozier and Arey (1919; Arey and Crozier, 1921) reacted on the basis of dermal sensitivity, with a lack of mantle eyes and no photic mechanism in the tentacles. In other species of this same genus and in related onchidiids the mantle is set either with short tubercles bearing eyes or with eyes embedded in the general tissue, these eyes having an inverted retina, a huge lens, and a mechanism allowing muscular accommodation.

Pelagic heteropods such as *Pterotrachea* have even more peculiar visual organs at the base of the tentacles (Fig. 14-7). A relatively enormous spherical lens is supported a short distance inside a hemispherical cornea. Small groups of visual cells lie at and near the bottom of an elongated cone extending inward from the lens; some of these groups are on projecting ridges, and the term "ladder retina" has been applied to the mechanism. Hess and Gerwerzhagen (1914) stimulated the eye electrically and found muscular movements that shifted the lens with respect to the retinas, presumably as a mechanism allowing accommodation. Whether the minute retinas on the various ladder steps allow use of the eyes as sights is not known.

*Cephalopoda.* Cephalopod eyes have been studied extensively because of similarity in form to that of the vertebrate eye. Krohn (1842) and Langer (1850) pointed out that good muscles are present, allowing accommodation; Beer (1897) investigated this aspect quantitatively and noted that no change in lens curvature occurred but that the lens itself was shifted with respect to the retina. In most cephalopods the eyes are disproportionately large; in *Sepioloa*, for example, a specimen weighing 3.5 g had eyes each weighing 0.9 g. At rest the focus is correct only for near objects, and accommodation is thus negative. Von Pflugk (1910) confirmed these conclusions in *Octopus*, finding the animal myopic by 6-10 diopters. Hensen (1865, 1866) made comparative studies among the cephalopods and considered the pinhole eye of *Nautilus* (Fig. 14-8) to be degenerate in its lack of cornea, lens, pupil control, and internal hyaline media. Embryological development, however, corresponds to the initial steps in the formation of more complex cephalopod eyes.

The cephalopod retina is not inverted, as is the vertebrate counterpart, and the origin of the whole eye is quite different, but it is equipped with muscles for shifting the eyeball. Heine (1907) and Alexandrowicz (1927) studied accommodation possibilities by stimulating the eye elec-

trically, and others (e.g., van Weel and Thore, 1935, 1936) investigated astigmatism in relation to the slit pupil. Heidermanns (1928) concluded that *Sepia* accommodated well for distance but that this was the mechanism for distance estimation, since vision appeared to be monocular (with only slight overlap in the fields of the two eyes). The image is clear, and he believed it to be appreciated both in detail and in color. On the basis of conditioned responses, Buytendijk (1933) concluded, however, that the eyes were used more in avoiding danger than in finding food; reaction times were very long even when the animal was hungry. The form of the pupil, moreover, seemed governed to a large extent by emotional state. Van Weel and Thore found pupil-control centers in the optic peduncle and subesophageal ganglion but reported that, in an unexcited intact animal, pupil aperture followed light-intensity changes. Steinach (1901a,b), by contrast, concluded that the reflex movements to light were based on a dermal sensitivity in the tentacles, not in the eyes. Baglioni's experiments (1909) showed similar difficulty in blinded specimens and led to the conclusion that vision, touch (including water movements), and taste all acted together inseparably, so that delay in capture of food when use of the eyes was prevented did not represent an accurate measure of the function of the eyes alone. Chun (1903) commented extensively on the value of eyes to the deep-sea cephalopods in bathic levels, where light production is a characteristic ability. More direct and valuable evidence has been made available from study of electrical action currents from cephalopod eyes stimulated by light: in *Eledone* by Beck (1899) and Piper (1904, 1911) and in the squid *Loligo* by Therman (1940). Piper (1904) obtained a good spectral-sensitivity curve. Therman investigated the electrical aspects of dark adaptation and effects of drugs. There can be no question that the eyes of cephalopods are sensitive to light, but, as in the vertebrate, the central nervous system is so highly developed that simple responses are seldom elicited when a captive animal is examined as a whole.

#### ARTHROPODA

The development of obvious eyes, both simple and compound, among the several classes of arthropods and the evident responses to light observable among the multitudinous genera have served to draw attention away from a broader sensitivity of a lower order—a dermal photosensitivity, or a photosensory activity within ganglionic areas. That decapod crustaceans include such mechanisms has been pointed out by Prosser (1934), Welsh (1934), and Schallek (1942) for the sixth abdominal ganglion of the crayfish and by Hess (1938b, 1940) for *Crangon* and the spiny lobster *Panulirus*. In *Crangon* and *Homarus* all abdominal segments appeared equally sensitive to light, and on the basis of regional photosensitivity of the uropods, Hess concluded that "cell bodies of the

neurons which connect with the peripheral spines are sensitive to light and function as photoreceptors.”

Among the insects a dermal photosensitivity has been reported in the walking stick *Aplopus*, a true bug *Neides*, and a cockroach and as the photosensory mechanism of larval flies (dipterans), larval beetles, and ants. Oehring (1934) pointed out some of the difficulties in establishing the functions even of the eyes, since blinding *Chironomus* larvae by the use of opaque lacquer was unsuccessful unless the whole head capsule was covered; otherwise light passed through the pigmented surface of the epicranium and affected the eyes from within. Oehmig (1939) found the same true of caterpillars. Welsh (1937) indicated that the true photoreceptors in the head region of fly maggots were still unknown, since the sensory papillae previously credited as light-sensitive organs proved gustatory. Final identification of the photoreceptors came from extremely careful microdissection technics, in which Bolwig (1946) destroyed limited areas in living housefly larvae and located a clump of rounded cells which, when eliminated, completely blinded the larvae. In early first-instar larvae these cells were not fully developed, and neither was light sensitivity. By the second instar the cell group was well organized but not yet enclosed by other than soft tissues. Such larvae orient with amazing precision, apparently evaluating the shadow cast by their own translucent bodies. In the third instar, growth of the main section of the pharyngeal skeleton provides a pocket almost surrounding the sensory cells, and with age this pocket deepens. Early third-instar larvae orient with even greater accuracy than second-instar, but this ability falls off gradually, apparently as a result of increasing opacity of the body. Third-instar larvae, however, are the only ones that will follow a resultant path between two light sources illuminating them simultaneously.

Arthropod eyes are of two major types: ocelli and compound eyes. The literature on these organs and on the activities of arthropods following their stimulation is enormous. Degenerate forms are found in many groups, and major puzzles remain in phylogenetic interpretation. Apparently even the ocelli have mixed origins. From comparative embryological and neurological studies, Hanstroem (1926) concluded that ocelli included (1) the nauplian eyes of crustaceans, the ocelli of insects, the median eyes of trilobites, the ocelli of xiphosurans, and the eyes of pycnogonids—all arising from a dorsal ectodermal mass in the embryo; (2) the lateral (“secondary”) ocelli of modern arachnoids and all eyes of diplopods and chilopods—through degeneration from the ommatidia of compound eyes produced by the lateral ectodermal mass of the embryo; and (3) the ventral ocelli of trilobites and xiphosurans and the median (“primary”) eyes of eurypterids and arachnoids—arising from a ventral ectodermal mass of the embryo. For crustaceans, Hanstroem provided particularly detailed accounts (1931, 1934a) emphasizing the neural con-

nections. In many ways these papers supplement the survey of Claus (1891) on median eyes in crustaceans.

*Ocelli.* Among crustaceans the median eye is basically a double structure, but fusion may be remarkably complete. In forms that metamorphose it may disappear. The extreme instance of change is in barnacles: in newly hatched nauplii there is a bilobed median ocellus; at the metanauplian stage a pair of compound eyes are added; at metamorphosis the compound eyes degenerate or are extruded; but throughout the metanauplian stage the median ocellus remains unchanged; only at metamorphosis does it divide into two and do the separate parts migrate into lateral positions in the mantle between the scutum and the juncture of the rostrum with the lateral plates of the shell (Fales, 1928). Thereafter they are the only photosensitive structures of the adult.

In the branchiopod *Artemia* compound eyes are added to the median nauplian eye. But if the compound eyes are blocked from stimulation, essentially all normal adult responses to light remain (Lochhead, 1939). Exceptions to this are a loss of visual following of females by males and obliteration of a peculiar convulsive reflex when the animal is illuminated suddenly after a long period in darkness.

The ventral position of the median eye in *Branchipus* and *Artemia*, many copepods, some trilobites, and xiphosuran larvae suggests that inverted swimming may be an ancestral habit. Inverted swimming is characteristic of *Limulus*, *Branchipus*, and *Artemia*, and probably also trilobites. A median ocellus could be of value, whereas the compound eyes were directed toward the bottom rather than the sky. Persistent nauplian eyes have been described in some decapods.

Among spiders eight ocelli are common. Of these one pair is of far simpler structure, but often each is provided with a cup-shaped retina that can be moved within the body through the contraction of pairs of muscles. These simpler eyes are the "primary" ones, and their optic nerve fibers arise from the proximal ends of the receptor cells; no tapetum is ever present. The remaining ocelli are "secondary" eyes; they lack muscles and often possess a tapetum. Usually their nerve fibers arise from the distal ends of the receptor cells. The distribution of secondary eyes is subject to enough variation so that the configuration of the ocelli provides a valuable taxonomic character. Morphological studies of spider ocelli (Brants, 1838a,b; Bertkau, 1886; Widmann, 1908) have been extended in an attempt to learn fields of view, possibility of stereoscopy, and limits of resolution (Petrunkevitch, 1907; Mallock, 1924; Homann, 1928, 1931, 1934, 1947a,b). Unfortunately Mallock's calculations are based upon the outmoded Rayleigh criterion, and his conclusions concerning the maximum acuity possible are unrealistic. Whether the muscular movements of the retina permit the spider to keep prey in view without body movements is still an open question (Homann, 1947a).

In *Aranea*, at least, form vision was not demonstrated, but the ocelli permitted an absolute evaluation of light intensity, used by the spider in determining the nature of its activities.

The number and arrangement of ocelli in other arachnoids are highly variable. In Solpugida the primary ocelli are usually on a tubercle; two pairs of secondary ocelli may be present elsewhere on the body (Hilton, 1932b). In Pseudoscorpionida a pair of primary ocelli are usual; in a few genera a pair of secondary ocelli are found in addition (Hilton, 1931). Among scorpions the primary ocelli are best developed, but from two to five pairs of secondary ocelli may be present laterally on the cephalothorax to the rear of the primary pair. In Pedipalpida ocelli may be lacking altogether, but usually a median pair are present on bead-like elevations, and three pairs of secondary ocelli in more lateral positions (Hilton, 1932a). In Phalangida usually one pair of ocelli is found on a turret on the back (Hilton, 1932c). No correlation has been noted between predatory, scavenging, or parasitic habit and the number or arrangement of ocelli among the Acarina. In Pycnogonida two to four ocelli on a special eminence are usual, but some are eyeless, and others have accessory ocelli in addition.

Among centipedes (Chilopoda) there is a full range from eyelessness to as many as 40 ocelli in a group as an aggregate eye or compound ocellus. Most millipedes (Diplopoda) possess simpler ocelli or none at all. No responses are known to be mediated by the ocelli in either group; blind forms seem to react in the same way as do those with eyes, and dermal photosensitivity is the only obvious explanation (Plateau, 1887a).

Ocelli are found in many insect orders, the characteristic number being two or at most three (Homann, 1924). Klug (1831) and Imhof (1901a,b) catalogued their presence or absence. Link (1908a,b, 1909a,b) described their morphological features in hemimetabolous insects and the adults of holometabolous insects. Some possess a tapetum (Hess, 1920c). Those of adult dragonflies may show strong astigmatism (Tuempel, 1912). In the grouse locust *Acridium* the ocelli are dimorphic in that those of the female show a double curvature—like a bifocal spectacle lens—producing two images at different distances from the lens (Tuempel, 1914). No explanation is available.

Immature stages of holometabolous insects are often described as having ocelli. Hesse (1901a) distinguished between "true" ocelli (without crystalline cones) in adult insects and hemimetabolous types and "isolated ommatidia" with crystalline cones in larvae of Lepidoptera, Trichoptera, Neuroptera (*s. lat.*), some Diptera, and a few Hymenoptera. Since the larval eyes in these holometabolous insects contribute nothing to the adult eyes, their correspondence in structure should be regarded as analogous rather than homologous. Those of dipterous larvae were studied by Constantineanu (1930).

Demoll and Scheuring (1912) analyzed the visual fields of ocelli and compared these with the fields of the corresponding compound eyes. Since there is so much correspondence, they concluded that the ocelli must furnish supplemental information. Negative response to light appeared to be mediated through the ocelli of May flies (Alverdes, 1923, 1924, 1927). Dragonflies soar and land with reasonable precision when their compound eyes are blackened but their ocelli are left intact (Tirala, 1923). The well-developed ocelli of the cockroach *Periplaneta* seem somewhat more reliable than dermal photosensitivity in allowing establishment of conditioned responses to light stimuli (Brecher, 1929). In *Drosophila* the photokinetic effect of light stimulation appears stronger when the ocelli are blackened, but no orientation takes place if the compound eyes are covered and only the ocelli are exposed (Bozler, 1925). Evidence of ocellar function in ants seems conflicting: Forel (1904) believed the ocelli to be valuable for near vision and in dark cavities; Caesar (1913) considered them useful only in respect to far objects while in flight; Mueller (1931) found that orientation to light vanished if only the ocelli were exposed, but the number of ocelli exposed (if any one was uncovered) made no appreciable difference in the reactions mediated through the compound eyes; blinding all ocelli caused changes in response similar to those reported by Bozler (1925) for *Drosophila*.

Wolsky (1930, 1931a,b) tried to correlate the dioptric system of insect ocelli with the activities of the respective insects. In the honeybee the lenses operated at  $f/2.3$ ,  $f/2.6$ , and  $f/3$  in the queen, drone, and worker, respectively. But since the receptors are not at the focal point for distant vision, Wolsky concluded that no image could be interpreted through the ocelli. This corresponds to the calculations of Tuempel (1912) and the resolution estimate of Hess (1920c). Among Hymenoptera, Goetze (1927) suggested that the primitive number of ocelli was three, that they were usually larger in males than in females, and that degeneration was frequent among nonflying forms. In these, however, whenever compound eyes had degenerated to rudiments, ocelli often remained. Otherwise in flightless forms well-developed ocelli and well-developed compound eyes commonly went together.

Aggregated ocelli take the place of compound eyes in the males of *Xenos* (Strepsiptera), according to Strohm (1910) and Roesch (1913).

*Compound Eyes.* According to Hanstroem (1926), arthropod compound eyes all arise from a lateral ectodermal mass in the embryo. They are found in crustaceans, trilobites, xiphosurans, eurypterids, many fossil chilopods and diplopods, the "house" centipede *Scutigera* and a few related modern genera, and most insects (including the degenerate "aggregated ocelli" of Strepsiptera). More degenerate still are the lateral eyes of certain crustaceans, certain trilobites, the remaining modern arachnoids, and most chilopods and all diplopods. The steps whereby

the lateral mass becomes organized into a battery of ommatidia were followed carefully by Watasé (1890).

The normal compound eye has been termed an "omma" and dissected tangentially into a dioptric system and a layer of soft parts (the "ommateum" of Lankester and Bourne, 1883) or radially into ommatidia (Carrière, 1884). The dioptric system consists of a corneal component that is molted and regenerated. In some eyes each ommatidium is distinct on the outside, since the corneal lens bulges as a separate facet, either square or hexagonal in outline. In other eyes the outer surface is perfectly smooth. In a few the corneal lenses are slightly concave externally.

Below the corneal lens and the hypodermal cells that secrete it, the ommatidium has some additional dioptric parts. None of these is molted. The ommatidium is described as of "exocoene" type if there is an inward extension of the corneal-lens material—as in crustaceans, trilobites, xiphosurans, and beetles of the families Dermestidae, Elateridae, and Lampyridae. The ommatidium is of "eucone" type if special cone cells ("Semper's cells") secrete a separate solid body within themselves. Ordinarily this process leaves the cone-cell nuclei distal to the cone, and sometimes there is also an anuclear portion of the cone cells basal to the cone. Eucone ommatidia are characteristic of the insect orders Thysanura, Orthoptera, Homoptera, Neuroptera, Trichoptera, Lepidoptera, and Hymenoptera and of some members of Odonata, most beetles, and some nematocerous Diptera. The brachycerous dipterans are unique in possessing "pseudocoene" ommatidia, in which the cone cells secrete distal to themselves a fluid or paste extending to the corneal lens and supposedly aiding in the refraction of light. "Acone" ommatidia, in which the cone cells become translucent and refract light and occupy all the space between the receptor cells and the corneal lens, are characteristic of the insect orders Dermaptera, Heteroptera, some Odonata, some Coleoptera (chiefly the families Silphidae, Histeridae, Coccinellidae, and Curculionidae), and some nematocerous Diptera.

At the basal end of the dioptric parts of each ommatidium is a ring of receptor cells, or two rings, one distal to the other. Sometimes there is an eccentric receptor cell outside the ring but extending a terminal segment to reach toward the end of the crystalline cone in a position central to the ring of receptors—like a core to the group. More commonly there is no eccentric cell, and the ring of receptors secretes a translucent rod in the core position as a rhabdom bringing light energy to the basal parts of the receptors along the optical axis of the ommatidium. Two rings of receptor cells may be more primitive than a single ring.

Investing the ommatidium like a sheath is a pigment-cell mantle. Often the pigment closes in around the base of the crystalline cone, limiting the passage of light to a very small aperture (van der Horst,

1933). In eyes so limited there seems to be no possibility that an image can be formed at receptor level, so that detection of intensity differences seems to be all that the ommatidium can provide for. Compound eyes used at light intensities of the order of daylight operate in this way, with each ommatidium isolated from its neighbors, and any central picture of the outside world must be built by the nervous system from intensity information sent along the many individual optic nerve fibers.

Compound eyes used in twilight and night intensities have the pigment very differently distributed. Grenacher (1879), who discovered this difference, referred to the isolated ommatidia of a day-type eye as composing an "apposition" type of compound eye, whereas the lack of isolation in a night-type eye permitted light entering many ommatidial lenses to be refracted and fall on the receptors of a central visual unit; hence the night-type eye was a "superposition" eye. The ray paths were traced by Exner (1889a, 1891), and attention was drawn to optical inhomogeneity within the corneal lens and crystalline cone, so that the dioptric system functioned in a far more complex fashion than merely as a long lens. In 1890 Szczawinska described migration of the masking pigment in a single compound eye, permitting the same organ to operate as an apposition type by day and a superposition type by night. The same action was discovered in a wide variety of crustaceans and insects (Stefanowska, 1890; Exner, 1889b, 1891; Herriek, 1891; Kiesel, 1894; Parker, 1895).

Not until Perkins (1928) learned that pigment migrations in crustacean compound eyes were controlled by hormones released into the arthropod blood, called forth by a reflex stimulation through the nervous system, did the time course of events make sense. Later it was learned that inherent diurnal rhythms in hormone production complicate the picture still more (Bennitt, 1932a,b; Welsh, 1935, 1936; Jahn and Wulff, 1941). Moreover in decapod crustaceans two different sets of pigment cells are involved—a distal set and a basal set—and the receptors themselves contain a migrating pigment (Welsh, 1930). The literature on this subject has become quite extensive, but most of it centers on the hormonal aspects rather than on the effect on vision and reactions to light stimulation. In insects, moreover, there is evidence that local responses and reactions mediated directly through the nervous system may explain pigment migration without involvement with hormones (Demoll, 1911; Day, 1941).

In many arthropod compound eyes a white or yellowish pigment is contained in special cells associated with the basement membrane through which the optic nerve fibers pass from the ommatidia. This is a tapetum, and pigment-cell migrations may expose it at low light intensities, permitting radiant energy received by the eye to have a second—reflected—chance to affect the receptor cells. As such it becomes an "occlusible"

tapetum analogous to that in the eyes of such vertebrates as the fish *Abramis*. A tapetum associated with the basement membrane can be called a retinal tapetum, in contrast to an "iris tapetum" of reflecting pigments in cells distal to the receptors. Iris tapeta are known in various crustaceans and insects as reflecting caps to pigment cells surrounding the receptors. These caps can be shifted distally or drawn basally, and their position alters radically the outward appearance of the eye (Zimmermann, 1913; Welsh, 1930; Uchida, 1934). In terrestrial arthropods the iris tapetum may assist the organism by rejecting ultraviolet or infrared radiations that would produce excessive heating at the receptor level.

According to Notthaft (1881), each ommatidium operates on an all-or-none principle. Either a target is included in its visual field enough to stimulate the receptor system, or it is not. This dichotomy is certainly too severe a view, but under certain circumstances a close approximation is reached. If the visual field consists of upright dark bands alternating with pale (as in a caterpillar's view of trees against the sky) or consists of pale flowers against a background of dark foliage, relative movement between a compound eye and the complex target will sweep each feature of the target across one ommatidial field after another, inducing on-off responses in the receptor cells. The characteristic swinging of a caterpillar's head or the normal movements of a flying bee provide all the movement required for a pattern to produce flickering stimulation in each ommatidium. The compound eye seems particularly efficient in detecting flicker. As in the human eye, there is a close relationship between the maximum rate of alternation in which the visual mechanism can detect dissimilarities between the bright phase and the dark phase, and the intensity of illumination. Flicker-fusion curves, like visual-acuity curves, are essentially straight lines when plotted on a probability grid (Wolf, 1933a,b). This may be due to a normal distribution of sensitivities among the ommatidia. Or it may arise through the convexity of the compound eye in that, the more intense the stimulation, the more ommatidia not facing directly toward the target area are obliquely illuminated at intensities above their thresholds; higher intensities would then recruit more ommatidia. Crozier and Wolf (1939) believed that they demonstrated that the latter was the limiting factor in the convex eye of the crayfish *Cambarus*.

Two very different ranges of flicker detection were found in insects by Autrum and Stoecker (1952). In the fly *Calliphora*, the wasp *Vespa*, and the bee *Apis* response to flickering could be detected at rates as high as 200-220 per second. In the cockroach *Periplaneta* and the grasshopper *Tachycines* any flickering rate higher than 5 or 10 per second was apparently fused into a constant stimulus. These authors postulated that in the orthopterans an afterimage extended temporal summation, whereas in the fly and hymenopterans examined afterimaging was lacking, per-

mitting the eyes to follow flickering at much higher rates. Since the criterion for visibility of flicker was irregularities in the electroretinogram, the conclusion does not relate to the central nervous system but to events within the compound eye itself—perhaps unlike rates of recovery after photochemical bleaching of the primary photosensitive pigment.

Flicker detection involves discrimination between two unlike intensities of stimulation presented in succession. Presumably the problem is scarcely different from that of two unlike intensities presented side by side simultaneously. For flicker the arthropod eye has been tested at length in terms of the intensity differences required. For detection of movement in the visual field a honeybee at its optimum intensity must have one stimulus 25 per cent greater or less than the other for detection of a difference (Wolf, 1933a,b). For the fruit fly *Drosophila* the difference must be of the order of 225 per cent (Wald and Hecht, 1933; Hecht and Wald, 1934). For the human eye, for comparison, a difference of 1.5 per cent is entirely adequate in good illumination. Hence the visual field of the arthropod eye contains a gray scale with far fewer steps than are characteristic of the human eye. The range of sensitivity is seldom so great, and only a fraction of the 500 stepwise increments between black and white detected by the human eye can be distinguished by the arthropod.

The contention of van der Horst (1933) that each ommatidium of an apposition eye must operate as a unit like a photometer, without detecting any image, still leaves room for additional abilities. The receptor cells in each ommatidium may be of two or more physiological types. If their photosensitive pigments are unlike, the wave length at which maximum sensitivity is reached in one receptor population could be different from the wave length for maximum in another population. This would correspond to the rod-and-cone system of the vertebrate eye, and a Purkinje shift with major changes in intensity could be shown in the spectral-sensitivity characteristics. So far this duality of receptor system has been demonstrated only in the fruit fly *Drosophila* (Fingerman, 1952; Fingerman and Brown, 1952). A neural basis for utilization of nerve impulses from separate receptor populations was described in dipteran eyes by Cajal (1909). Sánchez (1922, 1923) extended Cajal's findings into a theory to explain hue discrimination among arthropods, using a mechanism similar to that postulated by Young and Helmholtz for the vertebrate eye. No evidence has been offered to substantiate Sánchez's proposed differences among the receptor populations. But then no physiological differences have been found to have anatomical or chemical counterparts among human cone-cell populations in a color-sensitive eye.

Many arthropods do show definite hue discrimination. A peripheral basis for this ability was discovered by Graham and Hartline (1935) in the xiphosuran *Limulus*, even though no hue discrimination seems present

in this animal. Graham and Hartline measured the photometric intensity of light stimulus required in various wave-length bands to elicit the same nerve-impulse response to a flash of fixed duration. For any single ommatidium the maximum sensitivity fell between 520 and 530  $m\mu$ , and no Purkinje shift could be demonstrated by varying the intensity over a range of 1-100. Each ommatidium was remarkably constant in the spectral-sensitivity values obtained in repeated trials, but each ommatidium also differed markedly from others. When the several curves for a number of ommatidial action spectra were matched at the point of maximum sensitivity, it became clear that some ommatidia show more response than others to shorter wave lengths and correspondingly less response to longer wave lengths. Even two different ommatidial-response systems would make possible the discrimination of violet from red light, and three would permit full color vision—if the central nervous system were organized to utilize their unlike spectral sensitivities. In *Limulus* the central nervous system seems to lack this necessary organization. But a relatively minor change at the nervous level could provide for color vision in an arthropod sensory system.

The dimensions and divergence of ommatidia are subject to wide variations. Ommatidia facing downward commonly are relatively shorter and have larger lenses than those facing upward. Usually they diverge from one another more strongly. To a degree this is evident from a close inspection of the large compound eye of a dragonfly, as was pointed out by Ashton as long ago as 1840. But when sections are cut through the eye so that ommatidial axes can be plotted carefully [as by Baumgaertner (1928) for the honeybee eye], it is seen that in particular directions divergence between ommatidia reaches a minimum. For *Apis* this permits maximum acuity to lie in a plane inclined  $65^\circ$  to the sagittal, and in this plane only in an arc from  $47^\circ$  behind the anterior limit of the eye to  $49^\circ$  ahead of the posterior limit of the eye. Not only do the ommatidia diverge to different amounts, but each ommatidium appears to be compressed in its pigment-cell sheath—not in the corneal lenses or crystalline cones. Hence the angle between ommatidia is regularly greater in the transverse plane of the head than in the frontal plane, with a ratio of difference reaching about 2/1. Yet the radius of curvature of the bee eye is smaller in the transverse plane than in the frontal, with a ratio near 2.5/1. The whole compound eye then shows an astigmatic character to its visual-acuity possibilities. These morphological features are paralleled by differences in behavior depending on the direction in which the compound eyes are stimulated.

Autrum (1949) has generalized to the effect that, in all insects that fly well, the angle of view of each ommatidium in the horizontal direction is about twice that in the vertical. Hence a target remains for a longer time within the visual field of an ommatidium if it is moving horizontally;

summation can permit its detection at a lower threshold level than would be found in the same target moving vertically in the visual field. For the fly *Calliphora* electroretinograms bore out this relationship. It must be regarded as an astigmatism of the individual ommatidium.

The extreme of difference between ommatidia in a compound eye is met in some May flies (Zimmer, 1897; Priesner, 1916; Grabenhorst, 1930), in the dipterans *Bibio* and *Blepharocera* (Kellogg, 1898; Dietrich, 1907, 1909), and in the aleuronid bugs (Weber, 1934). In most of these the phenomenon is restricted to the male, but in *Blepharocera* the disparity in ommatidial dimensions is characteristic of part of the female population. In these arthropods the upper portion of the compound eye forms a sort of "turban," with elongated ommatidia having only slight angular divergence; the rest of the eye is of shorter ommatidia, diverging more markedly. Kellogg observed that the females with divided eyes also had piercing mouth parts and bloodsucking habits, whereas females with normal eyes (all ommatidia with the same-sized facets) lacked mandibles and fed on nectar. He pointed out further that among the crustaceans illustrated by Chun (1896) were several showing major differences in ommatidial dimensions, and that all of these with "divided" eyes were predators. May-fly eyes, by contrast, seem to be adaptations permitting the male to be the aggressor in twilight mating dances. The male detects a female against the dim light of the sky above in time to seize her and carry her off.

A very different interpretation for divided eyes was offered by Rádł (1901, 1902). In his view the two parts are of separate embryological origin, and the phylogenetic dissimilarities postulated led him to draw up a "duplicity theory" for the arthropod compound eye. Zavřel, studying various dipterans (1907), extended the hypothesis into a "triplicity" theory. But later workers have not supported either of these ideas (Dietrich, 1909; Priesner, 1916; Grabenhorst, 1930).

By sectioning arthropod heads through the compound eyes, it is possible to demonstrate which ommatidial axes converge toward those of the opposite eye, permitting a binocular field (Demoll, 1909c). Another technique, more satisfactory in some respects, is to examine the living eyes for directions from which a false pupil can be seen in each. The false pupil consists of a group of seven or more ommatidia facing directly enough toward an observing eye or camera lens so that light entering them from the direction of the observing eye or lens passes down the pigment-cell sheath and is absorbed by the pigment and receptors. It is evident as a dark spot that migrates in position according to the position of the observer. In a cylindrical compound eye, such as that of the ghost crab *Ocypoda*, the false pupil seen in horizontal directions is a vertical line the length of the cylindrical portion of the eye. Binocular fields are common among predaceous crustaceans and insects and appear to be

important in the capture of prey. Distance estimation is evident in both naiad and adult stages of odonate insects (Demoll, 1913; Baldus, 1924, 1926), robber flies (Melin, 1923), and tiger beetles. Originally it was believed that instinctive reactions led to the capture of prey when ommatidia whose axes intersected at the correct distance were stimulated simultaneously and symmetrically. But revision of this view is necessary since partially blinded odonates and tiger beetles will adapt their behavior to approach prey monocularly, pivot, and seize at the appropriate instant (Baldus, 1924, 1926; Abbott, 1949).

Under normal circumstances the various areas of a compound eye apparently serve definite reaction patterns. Painting over one eye or symmetrical parts of both often leads to postural modifications if the animal stands still or to circus movements if it progresses. Descriptions of these modifications are included in a great many texts as evidence for the "muscle-tonus" theory of animal behavior. Unfortunately the observers did not continue their experiments long enough to discover what remarkable modifications can be made by the arthropod nervous system, gradually eliminating the postural abnormalities and circus movements and achieving remarkably normal responses. Hence ommatidia that normally serve certain reflexes can take over for others that are blinded, if the central nervous system is given time to make the adjustments (Rabaud, 1921, 1925).

Among crustaceans, trilobites, xiphosurans, and the ametabolous and hemimetabolous insects, compound eyes grow at each molt. The process has been followed in one instar after another of the praying mantis *Sphodromantis* (Sztern, 1914; Przibram, 1930; Yamanôuti, 1933), the cockroach *Blatta* and walking-stick insect *Dixippus* (Przibram, 1930), the back swimmer *Notonecta* (Bernard, 1934), and various other insects and crustaceans (Bernard, 1937). New ommatidia are added along one or more margins, and previously formed ommatidia enlarge. Thus in *Sphodromantis* the number of ommatidia rises from 3144 at hatching through 10 molts to 8107. *Dixippus*, by contrast, adds no ommatidia, and the total increase in dimensions is 126 per cent, with a doubling of eye area from birth to maturity.

The development of the compound eye appears to depend upon normalcy of the supra-esophageal ganglion. Damage to this ganglion usually results in failure of the eye to differentiate. In *Drosophila* the genetic degeneration of the eye is through factors acting on the eye itself, not indirectly through the ganglionic background (Richards and Farrow, 1925). Degeneration of the compound eyes in cavernicolous arthropods and deep-sea crustaceans is common and apparently follows a genetic course influencing the eye itself. The literature on these degenerate eyes is quite large (Hanstroem, 1929). Some abyssal forms show hypertrophy of the compound eyes rather than degeneration, and this is believed to be

related to dependence upon light production by mates or prey for finding these important neighbors in the darkness of the depths (Chun, 1903). Beddard (1884), while reporting on the isopod crustaceans collected by the *Challenger* expedition, believed that there was a close relationship between depth and the degree of degeneration of the compound eyes, but unknown features of the abyssal natural history of each species make generalizations highly unsatisfactory.

Regeneration following injury to the compound eyes seems possible in decapod crustaceans, although the regenerated part is not an eye but an antenna-like organ. Regeneration of ommatidia appears to have ended with the trilobites. Using as a criterion the failure of ommatidial areas surrounded by scar tissue to line up in facet pattern with the rest of the eye, Isberg (1917) concluded that formation of new ommatidia was possible in this extinct class.

Often a relatively flattened compound eye conceals externally the extent of its visual field. Marginal ommatidia may be aligned with their optic axes almost tangential to the surface of the body. Only ideally is the external surface hemispherical and the ommatidial axis aligned in a truly radial direction. As a result, it is usual for the ommatidial axis to meet the corneal surface obliquely. To put it another way, it is unusual for the ommatidial lens to lie at right angles to the ommatidial axis. As a result of this, the proportion of incident light that enters the ommatidial dioptric system depends upon the plane of polarization of the light. Sky light is characteristically polarized with reference to the position of the sun, and in xiphosurans and insects, at least, the compound eye acting as a whole can furnish special information to the central nervous system, giving the animal a sky compass.

Von Frisch, who reported the polarization-detection feature of honey-bee eyes (1949, 1950a,b), credited the discovery to Autrum, although the latter did not publish an account of it until 1950. Griffin (1950) and others have supported the conclusion from independent experiment, and Waterman (1950, 1951) has found its counterpart among ommatidia of the compound eye of the xiphosuran *Limulus*. In describing the hymenopteran sky compass, von Frisch made use of an octagon formed from pieces of Polaroid film cut in a symmetrical pattern of triangles. Held toward the sky, the two triangles on some one diagonal were darkest, and those on the diagonal at right angles transmitted most light; between them were intergrades in transmission. It was only natural that similarity should be suspected between the eight radially arranged pieces of Polaroid film and the ring of receptor cells in an ommatidium. Perhaps each ommatidium served as a separate analyzer for polarized light, and the different receptor cells sent nerve impulses in a pattern advising the central nervous system of unlike intensities received via the rhabdom. Wolsky (1929), who investigated the corneal lenses of land isopod crusta-

ceans with polarized light, had found no such analyzer function. And apparently none need be sought. The obliquity of the ommatidial axes at the surface of the convex compound eye serves adequately. Seemingly this furnishes the information needed by the central nervous system of xiphosurans, ants, and bees—at least—in their ability to align their courses according to compass directions. Only in the fly *Volucella* has a claim been made that individual receptor groups could detect the plane of polarization (Menzer and Stockhammer, 1951); whether this too is due to obliquity of ommatidial axes cannot be learned from the description given.

Anatomical studies of arthropod compound eyes have been very numerous, but certain publications contain large enough blocks of fundamental information so that they have become the classics of the field: Grenacher (1879), Notthaft (1881), Watasé (1890), Exner (1891), Hesse (1901b), Demoll (1910, 1917), Best (1911), Hanstroem (particularly 1926), and Bernard (1937). For the most part, these retain a general flavor. Other reports are limited more to special classes and orders within the phylum.

Of the crustaceans, decapods and stomatopods have compound eyes on stalks and make compensatory movements of the eyestalks when the animal or its visual field is rotated. Amphipod compound eyes are usually as unspectacular as those of *Gammarus*, but in one group (the Hyperina) hypertrophy has produced enormous eyes occupying a major part of the enlarged head (as in *Hyperia* and *Phronima*), and in another [the ampeliscids (see della Valle, 1888; Svensson, 1934)] the eyes are divided into separate parts, and some of these degenerate (as in *Haploops*); in cavernicolous types degeneration has often proceeded as far as complete eyelessness. The Mendelian genetics of pigment loss is known in one species of *Gammarus* (Allen and Sexton, 1920). Most isopods have relatively coarse compound eyes, like those of the terrestrial *Armadillidium*, *Oniscus*, and *Porcellio*. But shallow and deep-water species show a range of structure (Beddard, 1884), and abyssal forms may completely lack eyes. Degeneration among cavernicolous isopods appears to be much more variable within each species (Banta, 1921; Kosswig and Kosswig, 1936). In the females of *Cymothoa*, which are parasitic in the mouths of fishes, degeneration is progressive during each life history (Eggert, 1927). The sole cumacid studied (*Diastylis*) has a median fused compound eye composed of eight ommatidia, four from each original eye. Some other genera in this group have two eyes, a fused median structure, or lack eyes entirely; nocturnality is common, and mud burrowing is a frequent habit. Among barnacles, compound eyes form during the metanaupliar stage but, depending on the species, are either extruded to fall away or absorbed at the time of metamorphosis into the adult (Fales, 1928).

Among entomostracan crustaceans all gradations can be found between genera with a distinct pair of compound eyes and genera in which the

two are indistinguishably fused on the mid-line. The fused median compound eye of *Daphnia* consists of about 20 ommatidia and is somewhat unusual in that it can be rotated several degrees within the body through the action of a series of oculomotor muscles. Anostracan branchiopods such as *Artemia* and *Triops*, which swim inverted, depend enough upon visual cues so that behavior patterns change when compound eyes are covered or act without the supplemental ocelli (Lockhead, 1939). Hanstroem (1934c) summarized compound-eye development among branchiopods and was able to show a logical series from a stalked eye (in anostracan genera) through incomplete fusion (in *Lepidurus* and *Triops*) to complete fusion. This sequence matches habits closely; both anostracans and notostracans habitually swim ventral surface uppermost, whereas the rest of the branchiopods maintain the more usual orientation. Copepod compound eyes range from the median fused structure of *Cyclops* and *Calanus* through the four eye types on each individual of *Argulus* (various stages of degeneration) to the extremely aberrant single ommatidia of the corycaeids *Copilia*, *Corycaeus*, *Labidocera*, *Phyllosoma*, *Pontia*, *Pontella*, and *Sapphirina*. In the corycaeids each of the two forward-looking ommatidia includes a large protruding lens capable of forming an image on a small internal structure consisting of an additional lens (perhaps a crystalline cone), a short rhabdom, and a group of receptors. The whole mechanism is more of a sighting device than an eye; unfortunately nothing is known of its function. Ostracod compound eyes are commonly separate if a median ocellus is present, but fused if the ocellus is lacking. Some ostracods lack compound eyes entirely. The luminescent *Cypridina* has fully developed eyes.

Among trilobites compound-eye development ranged all the way from complete absence to rather large eyes comparable to those of the xiphosuran *Limulus*. In any single species a gradual increase in the number of ommatidia appears to be correlated with increasing size (Reed, 1898; Richter, 1922).

The compound eyes of *Limulus* seem unique among living arthropods in that no ganglionic tissue is adjacent to the eye, and it is possible to dissect out individual nerve fibers from individual ommatidia and on them to study the passage of nerve-impulse trains in response to stimulation of single units of the compound eye (Hartline, 1930).

Among chilopods only *Scutigera* and some related genera possess compound eyes. In *Scutigera* each eye consists of 100-200 ommatidia with crystalline cones and 10-12 receptors apiece (Hanstroem, 1934b) arranged in a double circle, as in the thysanuran insect *Lepisma*.

The structure of insect compound eyes has been the subject of detailed study. The most satisfactory accounts for adult insects are those by Eltringham (1919), van der Horst (1933), von Buddenbrock (1935c), and Tischler (1936). Dethier (1942, 1943) reported on the ommatidium-like

eyes of lepidopterous larvae; these organs were called "composite ocelli" by Landois (1866) but were thought to be ommatidia by Pankrath (1890), Redikorzew (1900), and Sánchez (1926). Corneli (1924) has identified corresponding structures in larvae of the hymenopterous family Tenthredinidae, and Constantineanu (1930), in some larval dipterans. Schmitt-Auracher (1923) reported pigment migration in ommatidia of *Euproctis* caterpillars. Werringloer (1932) has contended that the compound eyes of ants are unlike those of other insects both in histology and in embryology. Neither Schmitt-Auracher's observations nor Werringloer's contention has received support by later investigators.

Attempts to find phylogenetic relationships among insect compound eyes have led to papers by Chatin (1876, 1878) and Lankester and Bourne (1883). Patten (1890) tried to prove that the ommatidium was a modified hair-bearing "sense bud," but his whole idea was based upon false premises: that the nerve fiber entered the crystalline cone and that the cone was the photosensitive element (Patten, 1887b). Lowne (1884) also held this view, although he recognized that the diaphragming effect of the pigment cells (pointed out by Will in 1840, 1843) argued against it. Viallanes (1892) followed Schultze (1867) in maintaining that the cones were chitinous and nonnervous and that the retinula cells grouped around the end of the cone must be the photosensitive part. Some of these difficulties in interpretation were not fully cleared up until the studies of van der Horst (1933). Debauche (1942) suggested possible homologies between retinula cells and sensory elements of other receptor systems. The association between tactile hairs and ommatidia, pointed out by Hertweck (1931), lends itself to broad generalizations such as those of Patten and Debauche.

Except for specialization of "turban" eyes, such as were described above in May flies and some other insects, the normal number and arrangement of compound eyes in this arthropod class are two, one on each side of the head. In the whirligig beetle *Gyrinus* the eye on each side is completely divided, with one part facing into air, the other underwater and facing the bottom. Some other beetles have divided eyes, but the cause is obvious in each instance: excessive encroachment on the eye area of the head by enlargement of the antennal base.

Experimental study of insect vision as a means of learning how the compound eyes function seems to have begun with the work of de Serres (1813, 1814), who used black varnish to cover eyes and parts of eyes and observed behavior changes. De Serres also introduced the term "false pupil," although his description of it was not so complete as that of Ewing (1826). Dugès (1830) concluded that binocular vision was possible in insects, but how useful it could be was far from clear. Dor (1861) suggested that the more extensive visual field of even one compound eye might compensate for lack of accommodatory ability, so that, with many

optic units, the compound eye might provide for appreciation of distance. He saw no significant difference between the central difficulty of summing information from 12,000 lens-equipped eyes and the difficulties for man's brain to correlate impressions from two. Yet Plateau (1888) was unable to find reactions to moving objects beyond a few centimeters and concluded that all compound eyes were hopelessly myopic. Only when the angular size of objects in the visual field and the angular divergence of ommatidia were appreciated was this difficulty cleared up. Yet Notthaft (1881), who provided the needed information, was not much more optimistic than Plateau, since he assumed that each ommatidium followed an all-or-none course in detecting targets.

Evaluations of acuity and binocular fields do check well with behavior (Zacharias, 1890; Luedtke, 1938, 1940), and the structure of the ommatidia is related to the amount of light available. For example, the tsetse fly *Glossina submorsitans*, which forages in open country, has more ommatidia per unit solid angle than *G. tachinoides*, which haunts shady thickets (Eltringham, 1936).

No confirmation has been found for the claim of Vigier (1904) that insects of rapid flight, such as dragonflies, have an accommodatory mechanism consisting of elastic and extensible parts represented by tracheae and myofibrils. According to Vigier, contraction of the myofibrils diminishes the curvature of the eye, and elasticity of the tracheae provides force for recovery. What good these mild gross changes would do for the compact ommatidia is hard to visualize. Any flexing would occur at the boundaries of facets rather than change the curvature and focal length of each cuticular lens. Vigier's view seems to depend upon the reality of image formation at receptor level, and this is lacking in the apposition eyes characteristic of fast-flying insects.

Controversy continues as to whether degeneration of compound eyes precedes or follows adoption of a lightless habitat (Pike, 1943). Commonly cave-dwelling species belong to genera in which considerable variation is known in the eye development of species living in illuminated habitats. Reed (1898) concluded that blind trilobites included both adaptive and degenerate types. In blind shrimp investigated by Neher (1901), the young had far greater eye development than the adults, and neural connections never degenerated. Kapterew (1912) found no irreversible loss of eye pigmentation in generations of *Daphnia* reared in the dark, but depigmentation of his initial light-adapted strain began after 12 days in the dark; in succeeding generations depigmentation (apparently by phagocytosis) began 4 days after birth. Tschugunoff (1913) noted stages of eye-pigment degeneration in the dark for the crustacean *Leptodora*. Genetic lack of eye pigment was suspected in an amphipod (Gegenbaur, 1858) and the daphnid *Simocephalus* (Banta, 1921). Definite differences in light reactions correspond to eye mutants in the moth

*Ephestia* (Klingebeil, 1938) and the fly *Drosophila* (McEwen, 1918; Brown and Hall, 1936). In the silkworm *Bombyx* different racial strains respond differently to light stimulation, although no basis for this has been found in the eyes (Waitzinger, 1933).

#### ECHINODERMATA

Langeloh (1937) has contributed the only account of light responses in crinoids; in *Antedon* he found the whole upper surface of the body to be photosensitive. Sudden illumination produced contraction of the longitudinal musculature, but shadowing had no apparent influence.

*Asteroidea*. Haeckel (1859) and later workers (see van Weel, 1935; Smith, 1937) described the compound ocelli at the tips of the arms of such starfish as *Asterias*, *Asterina*, and *Asteracanthion*. Wilson (1860) reported movements in the whole ocellar area which changed their positions in *Solaster*. Jourdain (1865), who distinguished between idioscopic (image-forming) eyes and photoscopic (responding to intensity and perhaps also direction) eyes, indicated that, although starfish ocelli appear capable of image formation, the relations of the parts merely provided concentration of light on the sensitive cells below the lens. Hamann (1883a) went farther in claiming that the ocelli were not light-sensitive at all. Jennings (1907), however, reported that *Asterias* in righting itself always turned toward the side away from lateral illumination and that, if prevented from escaping from a flat surface, it turned its arm tips away from the light. In a vessel with black sides it moved to the sides even when this involved approaching the light source, thereby suggesting vision of some sort. Bohn (1908) and von Frisch (1909) extended these studies somewhat, reporting movement into the shadow of a screen even when the shadow initially did not cover any part of the body.

Working with *Echinaster*, Cowles (1909, 1911a,b, 1914) noted that light stimulates movement toward increased illumination, a response that is more rapid when the animal is intact and slower when the tips of the rays are amputated. He concluded that the integument or the branchiae and tube feet were very sensitive to changes in light intensity but that the ocelli were important auxiliaries in oriented responses. *Echinaster* responded to a wide assortment of mixed wave lengths (Cowles, 1911b), with greater precision of orientation shown when a ray was pointed directly toward a source of light. Plessner (1913) found with *Asterias* and *Solaster* that the ocelli enabled a starfish to direct its movements toward a slit of light or a dark object and that their destruction eliminated these responses, although the general dermal photosensitivity allowed reaction to change in intensity of light by variations in kinetic activity. MacCurdy (1912, 1913) with *Asterias* reported the exact opposite—that removal of the ocelli did not alter the ability of individuals to orient as normally as intact specimens. Just (1926, 1927)

experimented with *Asterias* stimulated simultaneously by two or more separate light sources and found a variety of responses in the same individual. Three different patterns emerged: (1) direct movement toward one source, (2) movement with no clear relation to any source, and (3) movement on the resultant between two sources as far as a critical point. This point having been reached, the animal either (1) angled obliquely toward one source, (2) was already on the line joining the sources and there made a 90° turn toward one source, or (3) had gone beyond the line joining the sources and thence made an acute change in direction of movement to move to one source.

The stereotropic orientation of starfish tube feet has been used as a test in connection with light responses. Thus *Astropecten* illuminated from below rather than from above failed to right itself unless the ray tips with their ocelli had been removed; this operation allowed the righting response at once (Wolf, 1925). *Asterina* showed positive response to light when the aboral side was up but negative when inverted (Kalmus, 1929). The significance of these findings in terms of photoreceptors is far from clear. Probably van Weel's conclusion (1935) is most representative, namely, that, light sensitivity is characteristic of the ocelli, the terminal tentacles, the ambulacral tube feet, and the general body surface; that the entire skin area is able to inform the animal of shadows; and that some degree of directionality is interpreted by the central nervous system, leading to kinetic responses with definite orientation.

Detailed studies of the nervous connections in the starfish *Marthasterias* by Smith (1937, 1947) have yielded more information on the dermal photoreceptors. These seemed to be of a single kind, each a bipolar spindle-shaped cell 5–10  $\mu$  long, 1–1.5  $\mu$  in diameter, extending at either end into a long slender filament, and reaching the remarkable abundance of 70,000 per square millimeter. At the moment, information available on the nervous system does not indicate how far discrimination between physiologically different photoreceptors can be differentiated to inform the animal of its surroundings.

*Ophiuroidea*. Ophiuroids have been investigated, and their sensitivity seems to be dermal, general, and with no known photoreceptors. Cowles (1910) reported that *Ophiocoma* reacted negatively to brightly lighted fields unless some other factor changed the response. Thus these animals would not remain in a shaded portion of the aquarium unless they were in contact with the shading object. To put it another way, the shadow produced in a cavity has a different stimulating effect than a shadow alone. Cowles concluded that ophiuroids react to dark vertical walls even when they cast no shadow; this would indicate directionality comparable to that reported in asteroids.

*Echinoidea*. The earliest photosensory studies of echinoids appear to be those of von Uexkuell (1897) on *Centrostephanus*, in which he com-

pared latency to shadow reaction with latency to mechanical stimulation and concluded that, since the former extended from 0.50 to 0.85 sec, whereas the latter extended from 0.11 to 0.25 sec, two different sensory mechanisms must be involved. Later papers (Sarasin and Sarasin, 1885; von Uexkuell, 1900) employed the Ceylon species of *Diadema* in terms of light sensitivity and shadow reactions. Millott (1950, 1952, 1953), describing a Jamaican species of this genus, indicated clearly that the photosensitivity is dermal, with no specialized sensory structures found, suggesting that diffuse branches of the radial nerves might contain neuronal photoreceptors. A curious form of dark adaptation was reported in this species in that melanin-containing chromatophores in the skin concentrate their pigment as light intensity decreases, allowing light to pass between the chromatophores; hence a given stimulus is progressively more efficient in eliciting a response. Local light stimulation of the organism when in the pale dark-adapted condition also causes local expansion of the pigment granules. The response in all cases is a muscular movement of the long poison-bearing spines toward any region in which there is a sudden decrease in illumination. This may take the form of a shadow falling on the animal or merely a 40-watt incandescent lamp turned off from among a checkerboard of surrounding illuminating 100-, 60-, and 40-watt bulbs.

Cowles (1911a), working on *Toxopneustes*, found reactions to light and shadow in isolated pedicellariae, and these responses continued even when the pedicellariae were disconnected from the body; he concluded that "tonus centers" must lie in the tissues of the pedicellariae themselves. This he contrasted with von Uexkuell's findings on *Diadema*, in which severing the radial nerve connections to the spine base ended movements in response to light. Millott supports von Uexkuell in that the spines of *Diadema* are not photosensitive and the radial nerves are essential for mediating the light responses. Holmes (1912), working with *Arbacia*, reported similar movement of spines toward a spot of light thrown on the body surface; this response and the contraction of tube feet in light beams were not affected by cutting the nerve ring around the mouth, but after such surgery the animal was unable to crawl away from light into shadow (the normal reaction). Erection of spines in *Arbacia* as a shadow reaction seems strictly comparable to that reported in *Diadema*, but the response is much slower and less spectacular. Dubois (1913) gave an almost unbelievable account of sea urchin response to light for *Strongylocentrotus*, which commonly carries debris on its back by means of tube feet and pedicellariae. If light was directional and strong, Dubois found that these urchins picked up glass disks from the bottom of the aquarium and held them in such positions as to give maximum shade. Red glass was taken readily, but not green; circles and squares were handled indiscriminately; when the light directions were altered, the

urchin reoriented its body or the disks; and if a disk had a central transparent spot, the animal used another disk to shade the "hole" in its glass umbrella!

*Holothuroidea*. Hamann (1883b) illustrated what he believed to be light-sensitive eyespots in a sea cucumber. Clark (1898) traced the development of these sensory areas until they became red spots at the base of the tentacles. He found the arms to be photokinetic, responding negatively to a decrease in light intensity but not perceptibly to an increase; the entire body surface appeared to be sensitive to light. Olmsted (1917) was unable to establish whether the eyespots described had any light-sensitive function, though the whole body of *Synaptula* was photoreceptive. Even heads of this genus, severed just back of the tentacles, moved away from light; strong light on the headless bodies caused them too to turn away. Removal of tentacles (with eyespots) followed by unilateral illumination led to turning of the body in a direct line away from the light. Amputated heads lit by a spot of light, however, did not draw in the tentacles, as was characteristic of intact animals under such stimulation. Crozier investigated holothurian behavior in light (1914, 1917, 1920a,b) and noted that *Holothuria* moved with definite polarity (mouth anteriorly), whereas *Thyone* moved away from light like an echinoid—with any angle of the body in advance. Again the whole surface proved sensitive to light, with stimulation depending on the amount of light falling on the surface and not on the angle of incidence. Even isolated portions of the skin reacted to continuous light, thus indicating that light itself, rather than change in intensity, furnished the stimulus.

#### HEMICHORDATA

Dermal photosensitivity of *Balanoglossus* and its relation to the nervous system were investigated by Crozier (1917). Hess (1931, 1933, 1938a) found no specific photosensory organs in adults of *Ptychodera* and *Balanoglossus* but found that distribution of dermal photosensitivity corresponded to the distribution of neuronal photoreceptors below the skin. Hilton (1923) reported a possible eyespot at the tip of the preoral lobe in *Cephalodiscus* and *Rhabdopleura*. Larval hemichordates apparently bear eyespots with lenses (Spengel, 1893; Stiasny, 1914).

#### CHORDATA

Among members of the Urochorda, photosensory structures of ocellar type have been described in the free-swimming pelagic colonies of *Salpa* and related genera; no information on the use of these ocelli has been published. Grave (1920) and Mast (1921) studied the ocelli and light reactions of larval *Amaroucium* and found photosensitivity until the time of metamorphosis. Each ocellus consists of a series of three lenses, a cup-shaped mass of pigment granules, and a group of retinula cells.

Larval *Botryllus* has a similar ocellus (Woodbridge, 1924). Grave and Riley (1935) described the embryonic development of these photosensory structures. The so-called "ocelli" of adult *Ascidia* have proved to be insensitive to light stimulation (Hecht, 1918a), but neuronal photoreceptors within the oral siphon in the general region of the tentacles (1.0–1.5 cm below the "ocelli") are sensitive and cause immediate retraction of the siphon if illuminated. In other work Hecht (1918b, 1921c, 1926, 1927) studied photochemistry in terms of light responses in *Ciona*.

The "eyespot" at the anterior end of *Amphioxus* has proved to have no photosensory function. Along the nerve cord, however, are true eyespots that provide a sensitivity to light throughout the length of the animal. The degree of photosensitivity is roughly proportional to the population of these pigment-backed sensory cells. The most reliable accounts are those of Hesse (1898) and Parker (1906). Another possible type of photoreceptor has been found in *Amphioxus*, located dorsally along the nerve cord (Joseph, 1928). Known now as "Joseph's cells," these structures are unicellular and lack obvious pigment but otherwise correspond in detailed anatomy to the true eyespots.

#### SUMMARY

Throughout invertebrate phyla general photosensitivity is widespread. In many phyla specialized photoreceptors are added to the dermal receptor system, but the degree of organization of the specialized organs is not invariably correlated with obvious value in living habits. In many instances the nervous system appears unable to utilize fully the acuity provided by the dioptric apparatus. Adaptation to a range of light intensities, light reactions, and shadow responses seem to be functions primarily of neuronal photoreceptors and only secondarily of organized eyes. Photosensitivity of ganglionic nervous tissue has been found. It may be suspected of being a primitive characteristic.

Development of a functional lens, with or without an outer cornea, has occurred in annelids, mollusks, and arthropods. No homologies seem indicated. In each of these phyla are specialized types in which the eye can be accommodated. Compound eyespots, compound ocelli, and compound eyes have arisen among annelids, mollusks, and arthropods—in each instance with numerous basic differences in organization. In all an eye with increased resolving power has been achieved, permitting the animal to detect more details of its environment than is possible through dermal photosensitivity or any type of eyespot or eye less complex than a camera-style organ.

#### THE PHOTOSENSORY MECHANISM

Electrical records of action-potential changes in invertebrate photoreceptors were obtained first by Dewar and M'Kendrick (1873) with

various decapod crustaceans and by Beck (1899) with the cephalopod *Eledone*. Using similar methods, Piper (1904, 1911) obtained a spectral-sensitivity curve for the latter animal. Von Bruecke and Garten (1907) studied retinal action potentials in the crab *Cancer*. Hartline followed on an assortment of arthropods (1928), including the xiphosuran *Limulus*, and on the scallop *Pecten* (1938). More recently a single-ommatidium-single-nerve-fiber technic has been used with *Limulus* (Hartline and Graham, 1932a,b; Hartline, 1934, 1935, 1948; Riggs and Graham, 1940; Riggs, 1940; Hartline *et al.*, 1952). The advantages of the *Limulus* compound eye in such studies were pointed out by Graham (1932): the nerve fibers from sensory units (ommatidia) extend far enough from the eye before synaptic networks intervene so that electrodes can be applied to isolated single fibers. Various insects have been investigated using Hartline's technic (1928) or variations of it. From such studies have come many details concerning the relations between intensity and latency; spectral sensitivity; duration of stimulus and intensity reciprocity relations; effects of area of sense organ illuminated, of temperature, of dark adaptations, of diurnal rhythms, of recovery from brief illumination at high intensity; variations at threshold; and the like.

Latency has been evaluated in various invertebrates by observing kinetic responses of the whole animal: in *Gonionemus*, the crustacean *Cyclops*, the clams *Mya* and *Pholas*, the beetle *Popillia*, and the tunicate *Ciona*. Dark adaptation and light adaptation have been followed carefully in the protozoans *Volvox* and *Peranema*, the clam *Mya*, the slug *Agriolimax*, and various cephalopods through evaluation of iris movements or of pigment changes in retinal cells. Most precise measurements of dark adaptation are those with single sensory units of the *Limulus* eye by Hartline (1930) and Hartline and McDonald (1941, 1947).

Dark adaptation in crustaceans has been studied by measurements of rates of antennal beat in *Leptodora*, change in swimming direction in *Artemia*, and modifications of the shadow reaction in the barnacle *Balanus*. Inferences regarding dark adaptation have been obtained from electrical recordings of amplified action potentials from the eyes of the grasshopper *Melanoplus* and various moths. Modification of behavior, chiefly circus movements, has afforded clues to the same process in the drone fly *Eristalis* (*Eristalomyia*) and the large whirligig beetle *Dineutes*. Changes in response to flickering light gave information on dark adaptation in the honeybee *Apis* (Wolf and Zerrahn-Wolf, 1935), and alterations in shadow reaction allowed similar evaluation of the tunicate *Ciona* (Hecht, 1918b). A partial summary and chemical model for the process were furnished in a paper by Hecht in 1927, but the degree to which all of his analysis can be applied to sensory mechanisms alone (neglecting the nervous connections and effector system) remains to be demonstrated.

The question of spectral sensitivity in invertebrates has been studied

from a number of angles, of which that employing electrical measurements on optic nerve fibers seems by far the most reliable (Froehlich, 1913a,b; Graham and Hartline, 1935; Jahn and Wulff, 1946). Most difficult to evaluate are those based on photokinetic responses, since statistical numbers of animals and rigorous analysis have rarely been used, although interpretations have been drawn freely from small samples of doubtful uniformity. More valid, seemingly, are those studies based on pigment migration in the leech (Janzen, 1932) and in the eyes of crustaceans and insects. The kinetic response of many forms to ultraviolet light invisible to the human eye has led, on the one hand, to hasty conclusions that fluorescence provided full explanation and, on the other, to careful studies indicating that the moth *Plusia*, *Planaria* and other turbellarians, and *Daphnia* possess eyes responding to ultraviolet light itself—not by way of any fluorescent secondary effect—that they form images with it, and that dermal sensitivity in many instances extends also well into this part of the radiant-energy spectrum. Merker (1929, 1930, 1934) investigated also the transmission of the arthropod exoskeleton in the ultraviolet and measured a significant transparency as well as a relatively low level of fluorescence in normal intensities of these short wave lengths. The possible importance of ultraviolet vision in insects was investigated by Lutz (1924, 1933a,b) in terms of flowers and mates; Brues (1941) extended the study somewhat in terms of supposed mimicry in butterflies.

Early studies of spectral sensitivity were concerned primarily with what region of the spectrum provided most or least attractive radiation for orienting or photokinetic species (e.g., Graber, 1885). Thus Loeb and Wasteneys (1915a,b, 1916) concluded, on the basis of observations on hydroids, green flagellates, larval polychaetes, and larval barnacles, that invertebrates involved two types of photosensory substances, one with a maximum of absorption in the yellowish green near  $534\text{ m}\mu$ , the other with a maximum in the blue near  $477\text{ m}\mu$ . These results were in rough agreement with findings of T. W. Engelmann, Mast, and G. M. White. Similar but less uniform findings have come from work on *Volvox*, various green flagellates, turbellarians, the tube-building polychaete *Clitellio*, various leeches, the sea urchin *Psammechinus*, the clams *Mya* and *Pholas*, the snails *Limnaea* and *Littorina*, the squid, the octopus, and the protochordates *Amphioxus* and *Ciona*. Among the arthropods much of the controversy has centered around the possibility of color vision as distinct from intensity discrimination based on spectral sensitivities. The protracted arguments in print between the ophthalmologist C. Hess and the zoologist von Frisch gathered adherents in both camps—those following Hess in search for information showing that color vision did not exist, and those supporting von Frisch with data indicating color vision. Hess based his analysis primarily on demonstrating lack of a

Purkinje shift in the spectral sensitivities at high and low intensities and on comparisons with the sensitivity shown in Hering's famous evaluation of a totally color-blind man. Recent work in comparable technics includes that of Minnich (1940), in which reaction times measured for white light and for four regions of the spectrum with "equal energy content" were evaluated for 90 trials on 50 worms (*Clitellio*), with the conclusion that, since the light-adapted and dark-adapted worms showed maximum sensitivity in the same region of the spectrum, the photo-receptors must contain only one photosensitive pigment and hence the worms cannot distinguish different wave lengths. The opposite approach is typified by that of Liche (1934) on *Limnaca*, in which these snails were conditioned to a Y-shaped maze and distinguished between red and blue over a wide range of relative intensities as long as the eyes were present; brightness discrimination seemed to be a dermal function, but color vision, an optic possibility.

Color-vision studies have commonly provided contradictory results. Thus several have reported definite color selection by spider crabs and hermit crabs in the selection of "decorations" for the body, on the one hand, and of dyed snail shells, on the other. Others found no evidence to support these claims. Eyestalk compensatory reflexes have been used toward moving fields of vertical colored stripes in *Hippolyte* and *Carcinus*. Schlegtendal (1934) reported ability of the crab *Leander* to distinguish between red and blue and red and green and of *Crangon* to distinguish yellow from blue. *Daphnia* has been used widely for spectral-sensitivity investigations. Van Herwerden (1914) and Merker (1930) concluded that *Daphnia* could see in the ultraviolet as well as in the spectral bands visible to man; many of these workers credited the water flea with definite color vision. Related planktonic crustacea were investigated with reference to ultraviolet (finding responses following the Weber-Fechner logarithmic relation) by Erhard (1913) with colored papers in the Hering-von Hess tradition on *Simocephalus*. Scarcely any work has been done on the spectral responses of myriapods or arachnoids other than the studies on the xiphosuran *Limulus* by electrical methods and on *Lithobius* by Seharmar (1935).

Spectral responses of insects have been investigated primarily in relation to flower visits, to traplight collecting, or to destruction methods for pests. The pollination problem has an extensive literature (see work by H. Mueller, P. Knuth, von Kirchner, F. Knoll, and C. Robertson). More technical studies include those of Milne and Milne (1945) and Weiss (1943, 1944). In the caterpillar of the butterfly *Danaus*, Mayer and Soule (1906) reported no kinetic reaction in the spectral regions visible to man, but a strong positive response to ultraviolet. Janda (1931) noted pronounced negative response to ultraviolet (300-400  $m\mu$ ) in beetle larvae (*Anthrenus*), much less to visible light in human terms,

and no response to X rays, radium emanations, or  $\beta$  and  $\gamma$  rays of various hardnesses. Studies of the spectral responses and color vision of the honeybee have leaned on conditioned responses and observations in relation to natural and artificial flowers. Kathariner (1903) used colored feeding boxes; Lovell (1909, 1910, 1912) recorded the colors of flowers visited by marked bees and doubted the constancy of the bee to any hue. Turner's experiments (1910) with training bees, like those of von Frisch (1913-1923) and those of Ladd-Franklin (1913) and Hess (1909-1920), are strictly antagonistic in approach and results. Von Frisch's findings correspond, however, to those of Kuehn (1921-1927), Kuehn and Pohl (1921), Kuehn and Fraenkel (1927), Baumgaertner (1928), and others and fit well with Bertholf's spectral-sensitivity studies (1927, 1931). The drone fly *Eristalis* appears similarly to be able to distinguish colors (Ilse, 1949; Kugler, 1950).

Color response and spectral sensitivities of the honeybee have been studied together by Opfinger (1931) and Sander (1933). As in experimental work by Hess and others, these workers used colored and gray papers as test areas. None of these papers was calibrated in the ultraviolet region, which is so strongly stimulating to the honeybee, so that conclusions drawn from these studies are of doubtful value. Lotmar (1933) and Hertz (1937a,b,c) attempted to check on this aspect, but even through measurements of ultraviolet reflectance from standard papers (Heinig's series) they were unable to validate the earlier work. That the ultraviolet-sensitivity problem is important was emphasized by Stitz and Beyer (1927), who found changes in life history in the hive (apart from "harmful" effects) after installation of an ultraviolet source within it. Even the color of paint on the outside of a hive has occasioned debate: Some have argued in favor of contrasting hive colors to help bees find their way home more easily, whereas others have pointed out that, when bees swarm, they will either require a new hive of the same color (necessitating a large stock of empties on hand) or distribute themselves into the nearest hives of the same color.

Another approach to spectral sensitivity and color vision has been made through chromatophore changes observable particularly in mollusks and arthropods (Parker *et al.*, 1935). That in cephalopods is unique in being directly under nervous control, whereas that in the crustaceans appears regularly to be mediated by hormones in a neurochemical mechanism of considerable complexity. Color changes in response to environment and seemingly to vision have been studied also among the crab spiders (especially *Misumena*) since the first record by Angus in 1882.

Insect coloration has been subject to philosophical comment, such as is summed up by Poulton (1890) or more realistically by Judd (1899), Hesse (1933), and Carrick (1936). Matching of environmental colors has been studied at length in the walking-stick insect *Dixippus* (*Caurasius*)

without uncovering much information about visual mediation of the stimuli provided. Among the Lepidoptera correspondence between larval or pupal color and surroundings has been demonstrated and studied experimentally, again with few new data on the visual functions that may be involved.

Summation of flickering light and migration of eye pigments under illumination changes are two approaches to sensitivity which have yielded helpful facts. Ability to recognize direction of movement in a circumrotating visual field was evaluated crudely in two crabs by Clark (1896) and in the crayfish by Lyon (1899). Loeb and Ewald (1914) found good correlation between action of the hydroid *Eudendrium* and prediction on the basis of the Bunsen-Roscoe law<sup>1</sup> for intermittent illumination. Hecht (1921a) and Piéron (1925a,b) reported similar agreement in the clam *Mya* as long as the summation occurred within 8-9 sec. Folger (1926) and Hecht and Wolf (1932) extended the study further for *Mya*. Muskens (1904) reported a reflex in the pupils of the octopus as a reaction to a rotating visual field but did not follow his observation with intensity or spectral-distribution studies. Ewald (1913) used eye movements in *Daphnia* as a criterion with a rotating sector wheel to adjust relative intensities of two lights; hence he combined Talbot's law with the Bunsen-Roscoe effect. Responses of the crustaceans *Asellus* and *Cambarus* have been measured for flickering light without locating much new. Wolf followed these studies (1940) in terms of response to flicker when the crayfish was stimulated monocularly or bilaterally, and found that the more distant eye largely determined the threshold—that threshold is dependent upon the number of visual elements per unit of visual field undergoing transitions from an illuminated to a nonilluminated state (see also Holloway, 1916). Wolf (1937) and Wolf and Zerrahn-Wolf (1937) provided data also on the reactions of *Limulus* to flicker in terms of compound-eye area stimulated.

The more general work on flicker detection in insects includes a wide variety of studies on the water strider *Gerris*, the back swimmer *Notonecta*, dragonflies, butterflies, the flies *Archytas*, *Eristalis*, and *Pollenia*, the diving beetle *Dytiscus*, and the honeybee. In the dragonfly and honeybee the maximum flicker frequency detectable was in the same range (about 55 per second) as can be distinguished by the human eye, but contrast between light and dark phases had to be ten or more times as great to evoke a response in the insects.

The relation between eye-pigment distribution and light intensity as appreciated by the animal has been noted for some time. Rawitz (1891) noted it in the retinas of cephalopods; Arey (1916) studied it in the retina of the snail *Planorbis*. Szczawinska (1890) and Stefanowska (1890) reported these effects in a wide variety of arthropods. Decapods and

<sup>1</sup> More properly the Talbot-Plateau law for flickered light.

shrimplike forms have been studied extensively, largely in terms of hormonal interaction. *Gammarus* and the isopods *Ligia* and *Idotea* have been investigated also. A broader treatment among crustaceans is that of Bennett (1924), though his work was done prior to any appreciation of the hormonal mechanisms involved. Changes in insect eye color with light have been described and investigated, but although use of pigment migration as a tool for investigation of other visual physiology has been suggested, little has been analyzed except the mechanism itself.

Studies of visual acuity (resolving power) of the arthropod compound eye have been made from the standpoint of the dioptric apparatus, but more reliably from investigations of the activities and responses of intact animals; good agreement between these methods is characteristic of most papers. Kalmus (1937) tested the response of newly emerged nymphs of the walking-stick insect *Dixippus* to vertical stripes alternating light and dark. Hecht and Wald (1933, 1934) established a relation between illumination intensity and acuity in *Drosophila* and found at best a resolution  $\frac{1}{1000}$  as good as that of the human eye and  $\frac{1}{10}$  that of the honeybee. A number of insects were investigated for minimal angle for recognition of flickering light and for moving stripes. The close relation between acuity, intensity of illumination, and contrast discrimination enters into these papers. Evaluation of intensity discrimination separately was made for *Mya* by Hecht (1924a,b) and drawn together into a basic theory (Hecht, 1935a,b). Electrical-response studies of intensity discrimination in the xiphosuran single ommatidium were presented by MacNichol and Hartline (1948), and behavior measurements in the honeybee in this regard include the work of de Haan (1928a,b), Wolf (1933a,b), and Hoermann (1934). Hundertmark obtained values for the walking-stick insect *Dixippus* (1937b) and the nun moth *Lymantria* (1937a,c).

Practical values for these measurements of acuity and intensity discrimination are to be found in terms of form vision and pattern recognition. Von Buddenbrock (1935d) claimed the first description of compensatory eyed-tentacle movements to rotating visual fields for any gastropod (in *Helix*). Peckham and Peckham (1887) reported that there was definite identification by sight of the egg sac in the spider *Theridion* and that a male *Astia* (jumping spider) followed visually after a female even 10 or 12 in. away. Plateau (1887b) considered 1 cm as a maximum distance for form recognition in jumping spiders and wolf spiders (lycosids), with scarcely better in scorpions and far worse in all other arachnids. The Peckhams contested this interpretation (1894), citing many instances of prey and mate recognition at greater distances. Rainbow (1898) cited further examples of fair visual performance in arachnids. Baltzer (1924) believed that the appearance of prey must be meaningless to the web-spinning *Epeira*, but Peters (1932) demonstrated that running

out in this genus to remove prey must depend on optical cues. Heil (1936) presented evidence indicating both form perception and good distance appreciation in jumping spiders.

For insects Plateau (1885) held that reaction to light was entirely in proportion to intensity and not toward the shape of areas providing the light—this for the blowfly *Calliphora*, the drone fly, and two butterflies. Demoll (1913) and Baldus (1924, 1926), however, recorded fair form and distance perception in dragonfly naiads. Baldus found in unilaterally blinded specimens that binocular vision and size appreciation were both important in controlling the snapping reaction toward prey. Verrier (1929) noted that the leaf insect *Phyllium* had keen sight for moving objects and that the male located the female visually. De Lépiney (1928) found visual responses in caterpillars to vertical silhouettes, and Hundertmark (1937a,c, 1938) used this technic extensively for evaluating vision in these insect larvae. Goetz (1936), on the contrary, could demonstrate no response to marks in caterpillars of several butterflies, although larvae about to metamorphose were responsive to the general direction from which light came. Sight responses in other insects indicate many errors in behavior due to deficient vision, and false attempts to mate arising from this difficulty. Von Buddenbrock (1935a,b) used striped patterns to study form vision in the drone fly.

A considerable literature has developed on the visual finding of mates among the light-producing beetles known as "fireflies." Vision into the red and infrared among such fireflies is a noteworthy fact (Buck, 1937) in view of the spectral distribution of daylight. The only other infrared response cited in the literature as being mediated through the compound eye seems to be that of pigment migration in the ommatidia of the moth *Plusia* (Kiesel, 1894). Spectral characteristics of pigment migrations were studied in the visible by Collins and Machado (1935), with large effects reported in the ultraviolet but none in the infrared. Eltringham (1923), using very different experimental technics, demonstrated that different species of butterflies are quite unlike in their ability to see with red light, and this ability seemed related to the colors in the insects themselves. Other studies of pattern vision and form perception are primarily those concerned with the honeybee, especially on conditioned responses to marks placed outside the hive or in association with feeding stations. Similar technics used with the wasp *Philanthus* have been reported. Thus insects are not the reflex machines postulated by Loeb and others.

#### NOTE

In the foregoing an attempt has been made to furnish a brief guide to significant information on invertebrate photoreceptors. A digest of the extensive and scattered literature is impossible in the space available;

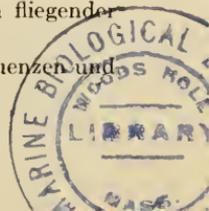
yet such a digest is needed, since there is no monographic treatment in print. The references cited have all been examined and abstracted. A more complete account of the subject will appear as a book, "Invertebrate Eyes and Photosensitivity," to be published about 1958.

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## CHAPTER 15

# Photodynamic Action and Its Pathological Effects

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*Introduction. Nature and mechanism of photodynamic action: Definition—Mechanism of photodynamic action—Alternative hypotheses—Other factors influencing photodynamic action—Nature of photodynamic agents—Nature of the substrate in photodynamic action—Effects of photodynamic action on the skin—Distinction between sunburn and photodynamic action. Investigation of photosensitivity diseases. Photosensitivity in animals: Syndrome of photosensitization—Classification of diseases involving photosensitivity—Diseases of Type I—Diseases of Type II—Diseases of Type III—Photosensitivity of uncertain etiology. Photodynamic action in human ailments. Addendum. References.*

### INTRODUCTION

The recognition of photodynamic action as a distinct branch of the biological effects of radiation has arisen from the observation by Raab (1900) that the killing of infusoria by acridine was greatly accelerated when the organisms were exposed to light in the presence of the dye. Raab's experiments were soon extended by Tappeiner and his associates, and the phenomenon was recognized as an oxidation reaction in which the necessary energy was supplied from radiation absorbed by the dyes introduced into the system. Jodlbauer and Busck (1905) showed that pruritus and edema were produced in mice and rats exposed to sunlight after injection with fluorescein dyes. These experiments gave a clue to the hitherto mysterious role of sunlight in certain skin diseases of animals, and Busck (1905) was apparently the first to follow this lead in his search for a fluorescent pigment in buckwheat as a cause of fagopyrism. Over the period 1930–1940 the systematic studies of Blum and his collaborators added considerably to our understanding of the fundamental mechanism of photodynamic action. The theoretical aspects of the subject and the existing knowledge of diseases caused by light were comprehensively surveyed in a monograph by Blum (1941a).

Since the publication of Blum's book there has been comparatively little further elucidation of the principles underlying photodynamic action, but much more is now known of the etiology of certain of the photosensitivity diseases, particularly those occurring in domestic ani-

mals. In this chapter the nature and mechanism of photodynamic action in biological systems are discussed, and the role of photodynamic action in various diseases, particularly those affecting domestic animals, is surveyed.

## NATURE AND MECHANISM OF PHOTODYNAMIC ACTION

### DEFINITION

At this stage some definition of what is meant by photodynamic action is desirable. When this expression was introduced (Tappeiner and Jodlbauer, 1904), it was not clearly recognized that the type of process discovered by Raab constituted only one of several ways in which radiation can influence biological systems. If precedent and use had not so firmly attached Tappeiner's term "photodynamic action" to this restricted field of photobiology, a less general description, such as "photosensitized oxidation," would now be preferable. It is therefore important to stress that by photodynamic action is meant an oxidation by molecular oxygen brought about in a biological system exposed to radiation through the agency of a fluorescent substance (the photodynamic agent).

The need for both light and an absorber of light was established in Raab's initial experiments. The need for oxygen was demonstrated by Straub (1904) and Jodlbauer and Tappeiner (1905) in systems such as the lysis of red blood cells or the killing of infusoria, and Blum *et al.* (1935), from experiments involving occlusion of the blood supply to an area injected with hematoporphyrin, have concluded that a supply of oxygen is necessary for photosensitization of human skin.

It is conceivable that some effects brought about by radiation may be similar to photodynamic action except that the final change is not oxidation but some reaction such as the disruption of a molecule. Such a process, if it were ever shown to occur, would fall outside the definition of photodynamic action.

### MECHANISM OF PHOTODYNAMIC ACTION

The resemblance between the conditions necessary for photodynamic action in biological systems and conditions pertaining in photosensitized oxidations *in vitro* suggested to Straub (1904) that the same fundamental mechanism was operating in both phenomena. More elaborate explanations have been advanced from time to time (Tappeiner, 1909; Clark, 1922), but Straub's original hypothesis, as developed by Blum (1941a), is the only one consistent with most of the facts. In accordance with this hypothesis the photochemical section of the process of photodynamic action may be conceived of as follows:

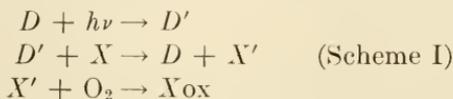
1. Energy in the form of radiation is absorbed by a molecule of the photosensitizing substance, which is thereby transformed to an excited

or activated state; i.e., the absorbed energy is retained in a form in which it can be transmitted to a second molecule with which the first may collide.

2. In collision with another molecule of lower energy status, the activated molecule transfers its energy and reverts to its former energy level.

3. The secondarily activated molecule (the acceptor) is then capable of combining with a molecule of oxygen—a chemical reaction in which it would not participate in its normal state.

This mechanism can be expressed in the following scheme, where  $D$  represents a molecule of the dye or other photodynamic agent,  $X$  is a molecule of the acceptor or substrate,  $D'$  and  $X'$  are the activated or reactive forms of these molecules, and  $X_{ox}$  is the final product.



The validity of this hypothesis can be best examined by considering how it complies with the known characteristics of photodynamic actions and with certain natural laws relating to photochemical reactions in general.

*Relation of Effective Light to Absorption Spectrum of Sensitizer.* A fundamental law of photochemistry is the Grotthus-Draper law, which states that only radiation absorbed by the reacting system is effective in producing chemical change. In compliance with this law the radiation producing a photodynamic effect (the action spectrum) should correspond exactly to the absorption spectrum of the photodynamic agent as it occurs in the system. In practice the comparison of action and absorption spectra is often difficult because the absorption spectrum of a photodynamic agent in a biological system may differ, through variation of physical conditions, from its absorption spectrum as measured *in vitro*. Screening or scattering of light by other substances (including the dye itself if present in too high a concentration) may interfere with the measurement. Methods of measurement also present difficulties, because usually the only practicable way to select portions of the spectrum is by use of filters transmitting light unevenly over a comparatively wide band of wave lengths and because measurement of the response in biological systems is rarely precise. Nevertheless in a few systems, with careful control of conditions, satisfactory agreement within the limitations of methods and materials has been obtained between action spectra and the absorption spectra of introduced photodynamic substances. These include tropic bending of roots of wheat seedlings sensitized with erythrosin (Blum and Scott, 1933), sensitization of human skin by hemato-porphyrin (Blum and Pace, 1937), sensitization of the skin of sheep by injected phylloerythrin (Clare, 1944), and sensitization of rabbits by hypericin (Pace, 1942).

*Energy Requirements of Photodynamic Systems.* The second law governing photochemical reactions is that of Einstein, according to which one molecule is activated for each quantum absorbed. This does not imply that every molecule that has absorbed a quantum of energy will react, since not all molecules will make collisions with other suitable molecules during their activated lifetimes. A quantum yield (i.e., number of quanta required per molecule transformed) of 1 is possible in the ideal case, and yields approaching 1 have been obtained in some reactions (Gaffron, 1933). In photochemical mechanisms involving chain reactions, quantum yields of less than 1 may be obtained, but, in general, yields are greater than 1 because a number of the activated molecules lose their energy before making an effective collision. A further complicating factor is the fact that sensitizer molecules may undergo recurrent activation, returning to a nonactivated state each time they have made an effective collision or have otherwise lost their energy. It therefore follows that quantum yields in a scheme such as that suggested for photodynamic action will be dependent on the concentrations of oxygen and of the substance undergoing transformation but are virtually independent of the concentration of the photodynamic agent, since the latter concentration influences not only the number of molecules capable of being activated but also the number of quanta that may be absorbed.

Using the hemolysis of red blood cells sensitized by dyes of the fluorescein group as their experimental system, Blum and Gilbert (1940b) estimated the number of quanta necessary to hemolyze a single red cell. In such a system the effective dye concentration is the amount of dye actually taken up by the cells, and the primary photochemical reaction involved is assumed to be the alteration or destruction of a substance within the cell membrane which affects the permeability or fragility of the latter. Evidence of such a process is the existence of an induction period of irradiation which must precede the onset of hemolysis. Within this system the number of quanta required per cell hemolyzed was found to be constant, of the order of  $10^{10}$ , for both rose bengale and erythrosin over a wide range of concentrations of the dyes taken up on the cell. A further observation in this experiment was that the number of quanta absorbed per dye molecule during the course of the reaction ranged from tens to thousands of quanta and was inversely proportional to the concentration of the dye. This is consistent with the concept of a photochemical process in which each activated dye molecule reverts, after either effective collision or dissipation of the acquired energy, to its initial state without destruction and is then capable of reactivation by further irradiation.

Another law that follows from the equivalence relation of Einstein is the Bunsen-Roscoe law, which states that, provided the degree of absorp-

tion does not change, the product of the duration of irradiation and the intensity of light gives a constant:

$$I \times t = k.$$

Attempts to determine whether this law applies to photodynamic action were made by Dognon (1928), using paramecia, and by Blum and Hyman (1939), who studied photohemolysis, but in both cases the results suggested some deviation from constancy for the product  $I \times t$ . Recognizing that a biological reaction such as photohemolysis proceeds in several stages, only one of which—the initial photochemical process—is subject to the Bunsen-Roscoe law, Blum and Gilbert (1940a) devised experiments to control such factors as the taking up of dye by the cells and the time occupied by the final process of lysis of the cells. Their calculations were based on the concentrations of dye taken up by the red cells, and the lysis stage was eliminated by measuring the least time of irradiation required to bring about hemolysis 24 hr after irradiation. Values for  $I \times t$  thus obtained were remarkably constant throughout any one experiment, indicating the relevance of the Bunsen-Roscoe law to this process.

An additional characteristic of photodynamic hemolysis which is consistent with the theory that it involves a simple photochemical reaction is that the initiation of hemolysis requires the same total amount of irradiation, whether this is applied continuously or intermittently. Blum and Morgan (1939) quote times of 330–367 sec required to produce 50 per cent hemolysis with intermittent light and dark periods and 350–380 sec with continuous illumination. Efimov (1923) had earlier obtained parallel results in experiments on the photodynamic killing of paramecia. Such results suggest not only that the process is of a simple photochemical type, not involving chain reactions, but also that it is irreversible.

*Influence of Temperature.* From their nature purely photochemical mechanisms are independent of temperature, since the energy of activation of the reacting molecules is acquired by the absorption of quanta of radiant energy and not through increase of kinetic energy of the molecules by heating. Some photochemical reactions may, however, also be thermal reactions, in which case they will show a temperature coefficient. If an initial photochemical reaction is followed by a thermal reaction, the over-all process may be similarly influenced by temperature. The photochemical reaction may also depend upon collision between particles, and the frequency of such collisions is influenced by change of temperature. In practice, therefore, it is not uncommon for photochemical reactions to show increases in velocity of about 10 per cent for a rise in temperature of 10°C.

If a temperature coefficient approaching 2 is found, it is most probable that the over-all reaction contains one or more stages that are thermal

reactions; if the reaction rate is not influenced by change of temperature, the reaction may be reasonably assumed to be free of thermal stages.

Negligible influences of temperature have been recorded by Hannes and Jodlbauer (1909) for the photosensitized inactivation of invertase and by Blum *et al.* (1937) for photodynamic hemolysis.

*Effect of Concentration of Reactants.* Determination of the order of a reaction by following the effect of concentration of reactants on the rate of formation of products is a common technique in studies of reaction mechanisms. A photochemical reaction such as that postulated for photodynamic action involves at least three steps—activation of the sensitizer, collision of activated sensitizer with substrate, and collision of substrate with oxygen—all of which may influence or determine the over-all reaction rate, and none of which can be studied independently. It is not to be expected, therefore, that reaction-order determinations will yield much information on the mechanism of photodynamic action.

*Significance of Fluorescence.* A property common to photodynamic agents is fluorescence. Since fluorescence represents the emission from activated molecules of energy previously absorbed during irradiation, this property is consistent with the concept of photodynamic action which has been outlined.

#### ALTERNATIVE HYPOTHESES

In developing the foregoing conception of the mechanism of photodynamic action, Blum (1941a) made a critical examination of various alternative hypotheses that have been proposed. Most of these involve a fundamental photochemical mechanism but differ from Blum's concept in method of utilization of the absorbed energy to effect the destructive changes. One older hypothesis of a different type—that the effects of photodynamic action are brought about by the light emitted as fluorescence by the irradiated dye—is mentioned here only because the writer has found it still commonly accepted among field workers concerned with photosensitivity diseases. Apart from its inherent improbability, since fluorescent light is not qualitatively different from radiation of the same wave lengths emitted by other sources, it is sufficient to state that it was tested and refuted by Raab (1900) himself. The chief alternative hypotheses are discussed in the following paragraphs.

*Theories Involving Activation of Oxygen.* A mechanism in which the sensitizer transfers its energy directly to oxygen, that is, a transposition of  $O_2$  and  $X$  in Scheme I, has been proposed (Kautsky *et al.*, 1933). These authors based their hypothesis largely on the effect of oxygen in quenching fluorescence and supported it with an experiment in which the sensitizer and the substance undergoing oxidation were kept apart by absorption on separate particles of silica gel. When the two sets of

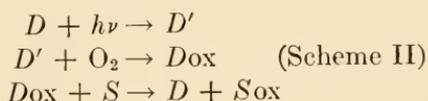
particles were mixed and exposed to light, the extent of the photodynamic oxidation was found to depend on the amount of oxygen admitted to the system. This experiment has been criticized by Gaffron (1936) because there was no certainty that exchange of sensitizer and substrate did not occur when the silica-gel particles were mixed.

The hypothesis of Kautsky *et al.* is, at all events, rendered untenable as an exclusive mechanism by Gaffron's work on the energy requirement of such a system. The energy of activation acquired by a molecule through collision with another molecule previously activated by absorption of radiation cannot exceed the amount contained in a quantum, which is in turn determined by the wave lengths of the radiation. It follows, therefore, that a reaction cannot be initiated either directly or indirectly by radiation unless the energy of activation of that reaction is less than the energy contained in 1 quantum of the absorbed radiation. According to calculations given by Gaffron (1935), the energy of activation required by Kautsky's hypothesis is 37 kcal, which is supplied only by quanta at wave lengths below 7620 Å. A photochemical reaction involving activation of oxygen therefore cannot proceed if radiation below 7620 Å is excluded. Gaffron (1935) found that photooxidation of thio-sinamine by bacteriopheophytin occurred in filtered light from which almost all radiation below 7600 Å was absent, indicating that activation of oxygen was not the mechanism involved in this reaction.

*Formation of Peroxides as an Intermediary Process.* Hydrogen peroxide is known to be formed when fluorescein dyes are irradiated in aqueous solutions, and such previously irradiated fluorescein solutions are hemolytic in the dark. However, it has been demonstrated that this effect is still produced after the hydrogen peroxide has been destroyed and also that the amount of hydrogen peroxide formed during photodynamic action is insufficient to account for the degree of hemolysis (Blum, 1935).

*Formation of Active Compounds of the Sensitizer.* Menke (1935) changed a dilute solution of sodium fluorescein into its photocompound by exposure to sunlight for 300 hr and found that this compound produced hemolysis in the dark to the same extent as fluorescein did in light. He also produced hemolysis in the dark with fluorescein solutions that had been irradiated for only 2 hr and concluded that photodynamic effects are due to the formation of minute quantities of photocompounds of fluorescent substances. Blum (1941a) has raised objections to such a mechanism on two grounds, namely, (1) that formation of such a compound in effective concentrations is unlikely during the course of photodynamic hemolysis, since other substances in a biological system would compete with the dye for oxidation, and (2) that such a mechanism would involve destruction of the dye after absorption of a single quantum, whereas absorption of many quanta by each molecule has been observed by Blum and Gilbert (1940b).

The second objection appears to assume the irreversible formation of a product of the dye. Blum (1941a) recognizes that this must be an oxidation product since it is formed only when the dye is irradiated in the presence of oxygen. If, however, the final lytic stage in the mechanism involves oxidation of a cell component, as required by the mechanism favored by Blum, an alternative process in which the photofluorescein effects this oxidation and is simultaneously reduced to its original form, thus becoming available for absorption of further radiation, would appear to be equally consistent with the known facts. Such a mechanism can be represented by the following scheme:



To determine whether such a scheme is valid and applicable to photodynamic actions in general, further information on the nature and chemistry of photofluorescein, and on whether similar substances are formed by irradiation of other photodynamic agents, is obviously required.

An observation made by Smetana (1938) in studies on the photodynamic action of hematoporphyrin on body fluids may be interpreted as evidence in favor of Scheme I. Smetana found that after prolonged exposure the uptake of oxygen gradually came to a standstill. Addition of more oxygen or hematoporphyrin did not appreciably speed up the reaction, whereas addition of further substrate promptly raised the oxygen consumption.

Another phenomenon noted by Smetana, namely, that uptake of oxygen continued, at a decreasing rate, for some time after the light was turned off, is not simply explained by either Scheme I or Scheme II.

When the various evidence discussed in this section is considered as a whole, the mechanism of photodynamic action put forward by Blum (Scheme I) is probably most in accordance with the established facts. Nevertheless, for the fluorescein system at least, Menke's photocompound suggests, in Scheme II, a possible alternative that warrants further study. It would seem that neither mechanism necessarily precludes the other; the over-all action may involve a combination of both processes (but see also Addendum to this chapter).

#### OTHER FACTORS INFLUENCING PHOTODYNAMIC ACTION

Investigation on the mechanism of photodynamic action has been assisted by the study of a number of other factors and conditions that influence it. These have been reviewed and interpreted by Blum (1941a) and will be referred to only briefly here.

*The Dark Action.* Many fluorescent substances will effect changes of the same type as those resulting from photodynamic action without expo-

sure to light, but the concentration required for this "dark action" is generally much higher than is needed if the system is irradiated. That photodynamic action is not merely an enhancement of this dark reaction is apparent from the demonstration (Blum and McBride, 1931) that the latter does not require molecular oxygen. The influence of this dark action must be eliminated (by choice of suitable concentrations) or allowed for in experiments on photodynamic action.

*Inhibiting Factors.* Since photodynamic action is an oxidative process, it is subject to inhibition by reducing agents, an effect well illustrated by Blum (1937) in experiments with sodium sulfite and thiosulfate. Inhibition by substances that combine with or precipitate the dye and thus prevent it from reaching the substrate was also demonstrated by Blum (1937). This gives an explanation of the inhibiting effect of serum, especially of the albumin fraction (Rask and Howell, 1928).

*Augmentation by Cyanides.* Studies on the effect of the addition of cyanides on photodynamic action have led to conflicting observations. Wohlgemuth and Szörényi (1933) found an increased oxygen uptake during photooxidation of serum when cyanide was added, and Bier and Rocha e Silva (1935) reported a similar effect in a hemolytic system within certain limits of concentration of cyanide, but Blum (1941a) found that cyanide, over a range of concentrations, did not significantly influence the percentage hemolysis time. The reported effect of cyanide was at first thought by Blum (1935) to support the participation of hydrogen peroxide in the mechanism of photodynamic action, the cyanide being considered to destroy the catalase that destroyed the peroxide. Blum calculated, however, that the amount of hydrogen peroxide which could be formed from the total volume of oxygen taken up during photohemolysis would not be sufficient to bring about that hemolysis. In his subsequent discussion (Blum, 1941a) the conclusion was reached that hydrogen peroxide is a minor product in photosensitized oxidation which may augment the hemolytic process, and that cyanide prevents catalase from suppressing the action of peroxide. The effect of cyanide on photodynamic action still appears to warrant further investigation. The subject has been revived by Rocha e Silva (1940) in his attempt to attribute photosensitization in cattle eating *Holocalyx glaziovii* to the enhancement of the action of phylloerythrin by hydrocyanic acid in this plant.

#### NATURE OF PHOTODYNAMIC AGENTS

Photodynamic agents represent many classes of substances with widely different molecular structure and properties. Most of them absorb in the visible range, but this is not essential; phenothiazine sulfoxide, for example, is a colorless substance that produces photosensitization in the cornea of calves (Clare *et al.*, 1947). The one property that appears to be common to all photodynamic substances is the power of fluorescence,

itself evidence of molecular structure capable of temporary excitation by absorption of radiation. Plant substances responsible for photosensitivity in animals must, of course, also be capable of retaining an activatable form during absorption from the digestive tract and passage through the liver to the systemic circulation. For this reason it seems unlikely that the pigment phycocyanin would be a photosensitizer in poisoning by certain algae, as suggested by Steyn (1943), since it would be expected to undergo fission into protein and a pigment phycocyanobilin (Lemberg and Legge, 1949), which because of its close similarity to bilirubin is not likely to be a photosensitizing agent.

From the Einstein equivalence law it follows that in an ideal system all photodynamic agents compared on a molarity basis would be equally effective, since each would bring about the oxidation of one molecule of substrate for each quantum absorbed. In actual systems photodynamic effectiveness is limited by a number of factors. Blum (1941a) presented evidence to indicate that the effectiveness of fluorescein dyes in photodynamic hemolysis largely depends on the extent to which they are taken up by the erythrocytes. The manner of uptake is also important, dyes that penetrate the cell being less effective than dyes taken up on the cell surface (Dognon, 1927). In the photosensitization of animals the apparent photodynamic effectiveness is conditioned by many additional factors, including the protective action of the outer layers of the skin, the stability and solubility of the agent in body fluids, and the ease with which it is excreted.

The occurrence of chlorophyll in green plants and the fact that one of its breakdown products, phylloerythrin, is the photosensitizing agent in an important group of diseases of animals have given rise to some misconceptions about the role of chlorophyll pigments in photosensitivity diseases. There is no evidence that chlorophyll itself can be absorbed from the digestive tract. In fact, in most animals it is readily broken down, and those derivatives such as phylloerythrin which are absorbed are ordinarily excreted through the biliary system. Photosensitization of herbivores by phylloerythrin occurs only as a result of derangement of the excretory mechanism of the liver, as will be apparent in the subsequent discussion of these diseases.

Nevertheless work in progress in the writer's laboratory (Clare and D. S. Letham, unpublished observations) has shown that leaves of *Panicum miliaceum*, dried in a current of hot air, contain a pigment that photosensitizes rats and guinea pigs. Severe effects have been produced in rats by 200 g of the leaves fed over 18 days, and a rat of 100 g body weight was killed by exposure to sunlight after a single dose of 10 mg of the pigment. There was no evidence of liver dysfunction in these animals. The absorption spectrum of this pigment suggests that it is a chlorin-type derivative. Discovery of it has raised the possibility that

some of the sporadic photosensitivity associated with plants such as legumes and rape may be caused by such a pigment. However, this pigment has not been detected in green leaves of *P. miliaceum*, and lambs dosed with preparations of the pigments in amounts much greater than they could obtain by eating the dried plant were not photosensitive.

It has also been found that rats can be photosensitized by a chlorophyll derivative present in sheep feces.

Such observations point to the dangers inherent in the use of rats and guinea pigs as experimental animals in testing for photosensitizing substances in plants or extracts of plants. The discovery of these pigments in *P. miliaceum* actually arose during feeding tests of plant extracts for a substance producing liver dysfunction, accompanied by phylloerythrin photosensitivity, in sheep and guinea pigs, so that the production of photosensitivity by the pigment present in these extracts proved quite misleading.

#### NATURE OF THE SUBSTRATE IN PHOTODYNAMIC ACTION

The identity of the substrate that undergoes oxidation—the molecule represented by *X* in Schemes I and II—has not been definitely determined. The substrate is either a substance essential for normal structure or metabolism of the cell or a substance that on oxidation gives rise to a toxic product, and since a wide variety of types of cells are affected, it apparently occurs universally in living matter. Most of the evidence available indicates that this substance is a protein constituent. The uptake of oxygen by photosensitized tissue extracts (Kosman and Lillie, 1935) or blood plasma (Smetana, 1938) appears to be determined by the protein rather than by the lipin fraction present in these systems. Smetana found that the action of light on a protein in the presence of a photosensitizer can completely destroy the antigenicity of the protein. Harris (1926) has shown that proteins, including albumins and hemoglobin, and the amino acids tyrosine and tryptophan undergo photo-oxidation in ultraviolet light and that this oxidation is accelerated by the presence of hematoporphyrin and other fluorescent substances. Of particular interest was his demonstration that gelatin, which does not contain tyrosine and tryptophan, was not affected by photodynamic action. Unfortunately the experiments of Harris do not clearly distinguish between effects of ultraviolet light and photodynamic action. Carter (1928) concluded that the presence of easily oxidizable groupings, such as those in oleic acid and acetaldehyde, was not itself sufficient to ensure photodynamic oxidation and that aliphatic compounds were not affected. The structure common to susceptible compounds appeared to be hydroxyl or amino groups attached to a benzene ring. Among amino acids, Carter and also Lieben (1927) recorded uptake of oxygen by histidine as well as by tyrosine and tryptophan.

From this work it appears highly probable that proteins are the oxidizable substrate in photodynamic action and that tyrosine, tryptophan, and perhaps histidine are amino acids affected within the protein molecule. Whether all proteins containing these amino acids are susceptible or whether certain proteins are specifically attacked, what is the nature of the products formed, and how alteration to the protein affects the structure or function of the cell—all these are questions that remain unanswered. The development of partition chromatography for protein chemistry may simplify the approach to these problems (but see also Addendum to this chapter).

#### EFFECTS OF PHOTODYNAMIC ACTION ON THE SKIN

The effects generally observed in photosensitized skin do not depend on the nature of the photodynamic agent. Local effects produced by injection of the agent are similar to the triple response described by Lewis (1927)—i.e., erythema followed by edema of the exposed area and the subsequent spreading outward of erythema, but not edema, into the surrounding skin. Intense itching accompanies these changes, and pigmentation of the skin is an aftereffect.

Most of these reactions are probably consequences of cell damage initiated by photodynamic action, but not peculiar to it, and resemble general inflammatory processes, the mechanism of which has been discussed by Menkin (1940).

#### DISTINCTION BETWEEN SUNBURN AND PHOTODYNAMIC ACTION

From comparison of characteristic features of these two conditions, it is apparent that the mechanism responsible for photodynamic action is fundamentally different from that operating in sunburn, since Blum *et al.* (1935) have shown that sunburn can occur in the absence of oxygen. The action spectrum for sunburn is limited to radiation shorter than 3300 Å, whereas most, if not all, photodynamic action spectra are of longer wave length, generally including visible light.

#### INVESTIGATION OF PHOTOSENSITIVITY DISEASES

Although there are obvious gaps in our knowledge of the fundamental processes of photodynamic action, certain inferences drawn from that knowledge are of value in formulating an experimental approach to the subject, particularly in its more practical aspect—the study of photosensitivity diseases. Such a study resolves essentially into proof that photosensitivity is occurring, the identification of the photodynamic agent, and discovery of the way in which it reaches the skin. Blum (1938, 1941a) has proposed three postulates that should be fulfilled before identification of the photosensitizing agent can be considered conclusive:

1. The subject must be shown to be sensitive to sunlight transmitted through window glass.

2. A photodynamic substance must be isolated which will produce this sensitivity to light when injected into experimental animals.

3. The action spectra for postulates 1 and 2 must be identical and should correspond to the absorption spectrum of the suspected photodynamic agent.

Sunlight is stipulated in the first postulate because the sun is the usual source of light to which skin is exposed and it is difficult to devise light sources that simulate sunlight in intensity and distribution of energy. Window glass, or a similar absorber of the erythema-producing radiation of about 3200 Å, is required to eliminate confusion with sunburn. Application of this test might have prevented a number of diseases involving skin lesions from being incorrectly reported as photosensitivity.

In the second postulate Blum has specified injection as the mode of administration. He recognizes, however, that in most diseases of animals the photosensitizer comes from a plant or other ingredient of the diet and that oral administration of the suspected substance is the ultimate test in such cases.

Injection tests with plant extracts should be regarded solely as a guide to isolation, and then only if chlorophyll or its degradation products, which are themselves photodynamically active, have been removed. The tendency has been to disregard as possible photosensitizing agents the chlorophyll derivatives obtained directly from plants, since there was no evidence that these pigments might reach the systemic circulation unless the excretory function of the liver was impaired. The photosensitization of small animals by chlorophyll-type pigments obtained from *Panicum miliaceum*, referred to earlier in this chapter, suggests that chlorophyll pigments should not be entirely neglected if extracts containing them are active on oral administration.

The third postulate is frequently difficult to establish experimentally because of the lack of convenient means of isolating various parts of the spectrum, the wide range of wave lengths absorbed by most pigments, the differences between absorption spectra of substances *in vivo* and *in vitro*, and the difficulty of evaluating the response in animals. Defining the action spectrum for a photosensitivity may assist in excluding a suspected photodynamic agent but can rarely confirm its identification.

A disease in which the determination of the action spectrum was useful in distinguishing between several possible photosensitizers is the keratitis of calves dosed with phenothiazine (Clare *et al.*, 1947).

An approach not specifically referred to by Blum is the examination of blood or tissue of photosensitive animals for photodynamic substances not normally present. Such examinations have contributed materially to the establishment of phylloerythrin as the sensitizer in the group of

livestock diseases in which photosensitivity is a consequence of liver dysfunction (Rimington and Quin, 1934; Clare, 1944). Certain precautions, however, are necessary in such an approach. The animals must be photosensitive at the time the sample is taken; some attempt should be made to relate the amount present in the blood to the amount needed to produce photosensitivity; and failure to detect a pigment does not eliminate it from consideration, since photosensitivity produced by injection of phylloerythrin may still occur, presumably through deposition of sensitizer in the skin, after the level in the blood has fallen below the detectable limit (Clare, 1944). Furthermore, as Blum has emphasized, the appearance of photodynamically active porphyrins in blood and urine of congenital porphyria and other conditions is inadequate grounds for the popular belief that these porphyrins are responsible for the sensitivity to light and skin lesions as seen in *hydroa aestivale*.

A further criterion that can be applied to distinguish between true photodynamic action and other effects of light, such as sunburn, is based on the requirements of oxygen in the former. By this means it has been shown that the dermatitis that affects some persons after treatment with sulfanilamide (Blum, 1941b) and the condition known as "urticaria solare" (Blum *et al.*, 1935) are not due to photodynamic action.

## PHOTOSENSITIVITY IN ANIMALS

### SYNDROME OF PHOTOSENSITIZATION

The symptoms and clinical signs observed in photosensitization do not depend upon the nature of the agent or the wave length of the light (provided the sunburn radiation is excluded), but they do vary with the intensity of the photodynamic action. Erythema accompanied by pruritus is commonly the first observable effect and may appear within a few minutes of the start of insolation. If the photoreaction is sufficiently strong, edema of the exposed area follows within a few hours, even if the skin has meanwhile been protected from exposure, and exudation of this edema through the skin may occur. Gravitation of the edema may result in swelling of areas that have not been exposed, such as the submandibular space and the legs. In animals such as sheep the ears swell and droop in a characteristic attitude. Necrosis and sloughing of the exposed skin may follow within a few days, the ears of animals being again particularly affected in this way. In cases of older standing in sheep, for example, the tips of the ears are often withered and curled up in a distinctive manner, and wool growth over the exposed areas is inhibited. Formation of suppurating sores in animals is, of course, a consequence of secondary infection of the damaged tissue, but it has occasionally given rise to an erroneous belief among workers with farm animals that the disease may be transmitted by contact.

In experiments with white mice injected with hematoporphyrin, Hausmann (1910, 1914) divided the effects he observed into acute, subacute, and chronic classes. In the acute form, produced when larger amounts of porphyrin were injected and the irradiation was intense, convulsions occurred, and the animals died within a few minutes. The acute reaction was evoked also by smaller dosage of light or sensitizer, but then the animals showed pruritus, erythema, and excitement, followed by weakness and coma, and died within a few hours. Subacute reactions consisted of erythema and edema, but the animal survived exposure for a considerable period, so that the aftereffects such as necrosis of the skin were observed. The chronic signs, consisting of necrosis and loss of hair some time after exposure, with only minor reactions during exposure, were the result of still lower dosage of light or porphyrin. Gray mice, for example, showed only the chronic effects.

Photosensitivity in human beings and agricultural animals is commonly of the subacute or chronic type under Hausmann's classification. Effects consistent with the less acute form can be produced experimentally in sheep, and such effects and even death after prolonged exposure can be seen in some cases of diseases such as facial eczema and hypericism. Death in these animals may frequently be the result of other lesions in the disease or of inability to graze normally rather than the effect of photodynamic action alone.

#### CLASSIFICATION OF DISEASES INVOLVING PHOTSENSITIVITY

The existing knowledge of the nature and mechanism of photodynamic action, incomplete as it is, has led to considerable progress in the elucidation of a number of pathological conditions involving abnormal reactions on exposure to light. In few of these diseases is the photosensitivity the only pathological manifestation; more commonly it is a consequence of other disturbances of bodily function, and frequently it is a minor aspect of the disease. The following classification of diseases involving photosensitization can be made, using as a basis the mode by which the photodynamic agent reaches the systemic circulation (Clare, 1952):

*Type I, Primary Photosensitivity.* In this type the photosensitizing agent is either a substance, not normally encountered in the diet, which is absorbed directly from the digestive tract and not completely excreted by the liver, or a substance introduced into the tissues of the skin by injection or contact. In animals there are two well-established diseases of this type due to poisoning by *Hypericum* species and buckwheat, and a number of other ill-defined photosensitivities caused by plants probably belong to this group. In human beings primary photosensitization has generally arisen through medication with fluorescent substances.

*Type II, Photosensitivity Due to Aberrant Pigment Synthesis.* The photosensitizing substance is a pigment, not normally found in animals,

which is produced endogenously by an aberrant metabolic process. Alternatively the aberration may be the excessive formation of a pigment ordinarily produced only in small, harmless amounts. In this category is the photosensitivity associated with congenital porphyria in cattle.

*Type III, Hepatogenous Photosensitivity.* The photosensitizing agent is a substance, normally absorbed and excreted, which is diverted to the peripheral circulation through failure of a liver excretion or detoxication mechanism. This type is represented by those photosensitivities in which phylloerythrin, a product of chlorophyll digestion normally excreted by the liver into the bile, accumulates in the systemic blood as a result of liver dysfunction. In most diseases known to belong to this type, the phylloerythrin excretion has been deranged by a plant hepatotoxin, but mechanical bile-duct obstruction may also be responsible.

This classification is proposed primarily to facilitate discussion of these diseases and to emphasize the very different etiologies of diseases in which photosensitivity can occur. It is possible that among diseases of obscure origin still further mechanisms will be found by which a photodynamic agent may reach the skin. Kidney dysfunction, for example, might lead to retention of a substance normally excreted in the urine, or disturbance of the selective permeability of the gut wall might allow the entry of a substance not normally absorbed.

#### DISEASES OF TYPE I

*Hypericium.* This disease, the oldest and best-known representative of the primary type of photosensitivity, occurs in sheep, cattle, goats, and horses through ingestion of certain species of *Hypericum*, principally *H. perforatum* and *H. crispum*. Černý (1911) extracted a red fluorescent pigment, hypericin, from *H. perforatum*, and a number of investigators subsequently demonstrated the photosensitizing properties of extracts containing hypericin (Ray, 1914; Horsley, 1934; Quin, 1933c). Pace and Mackinney (1941) resolved hypericin into a mixture of closely related fractions and presented evidence that indicates that these substances are partly reduced polyhydroxy derivatives of helianthrone. Pace (1942) obtained good agreement between the action spectrum for photosensitivity produced by oral administration of either hypericin or the plant itself and the absorption spectrum of hypericin.

*Fagopyrum.* Sensitivity to light of animals fed on buckwheat (*Polygonum fagopyrum*) has been reported frequently over the last 150 years, and this was in fact the first photosensitivity disease in which the lesions were attributed to photodynamic action (Busck, 1905). Evidence of the presence of a photodynamic pigment in buckwheat was obtained by Busck (1905), Öhmke (1908), and Chick and Ellinger (1941), and finally Wender *et al.* (1943) isolated three crystalline substances, all of which produced

photosensitization on oral administration to guinea pigs. Little is known of the constitution of these compounds, but Wender *et al.* suggest, from comparison of absorption spectra, that they may be related to the hypericins. The absorption spectra of the buckwheat pigments isolated by Wender *et al.* are consistent with observations on the action spectrum for fagopyrism made by Sheard *et al.* (1928), Mathews (1938), and Chick and Ellinger (1941).

*Photosensitized Keratitis in Calves Dosed with Phenothiazine.* An unusual form of photosensitivity which also falls within the primary type is the keratitis, or corneal opacity, which occurs in calves dosed with phenothiazine (as an anthelmintic) and then exposed to sunlight (Whitten *et al.*, 1946). Sheep are not affected unless large amounts of phenothiazine are given. Clare (1947) found that in both calves and sheep the sulfoxide derivative of phenothiazine is absorbed from the digestive tract and converted to phenothiazone, but in calves this conversion is incomplete, so that phenothiazine sulfoxide passes to the systemic circulation and hence to the aqueous humor. Phenothiazone, however, probably because it is conjugated as an ethereal sulfate, does not reach the corneal tissues. Phenothiazine sulfoxide was found in aqueous humor of affected calves and produced keratitis when injected into the anterior chamber of the eye of an undosed animal exposed to light. The action spectrum in these experiments was limited to the narrow range of wave lengths (up to 3600 Å) corresponding to the absorption spectrum of phenothiazine sulfoxide (Clare *et al.*, 1947).

There have been reports (Thorning *et al.*, 1942; Swales *et al.*, 1942; Britton, 1943) of photosensitivity of white skin and keratitis following administration of phenothiazine to pigs, another species in which phenothiazine sulfoxide is found in the peripheral blood (Clare, 1947). It is possible, however, that the phenothiazone contributes to the skin lesions in such cases, since even sheep will show a photoreaction of the skin if the skin is shaved and exposed to intense light (*ibid.*).

Apart from this hazard in the use of phenothiazine in calves and pigs, there appear to be no other reports of photosensitivity in domestic animals following medication with fluorescent substances.

*Other Photosensitivities of the Primary Type.* The photosensitization of rats and guinea pigs by pigments in dried *Panicum miliaceum* has been discussed earlier in this chapter, and since it has not been demonstrated in domestic animals, it need not be considered further here. Many other plants have been accused of causing photosensitivity, sometimes on sound, but often on slender, evidence. Because there is so little known of them, these cases will be referred to in an unclassified group later in this review. Nevertheless it is very probable that many of these diseases are of the primary type, especially some of the better known ones such as trefoil dermatitis, rape scald, and *Erodium* photosensitivity.

Finally, to complete the list of primary photosensitivities and in deference to the contributions by experimental animals to our knowledge of the subject, mention must be made of photodynamic action so frequently induced in such animals by injection of fluorescent substances.

#### DISEASES OF TYPE II

The only photosensitivity of this type among diseases of animals is that accompanying congenital porphyria in cattle reported from South Africa by Fourie (1936) and Fourie and Rimington (1938). There has been much discussion, which will be referred to in a later section, as to whether skin lesions seen in some human cases of congenital porphyria are due to photodynamic action by porphyrins, and Blum (1941a), with an impressive array of evidence, concluded that there was no experimental basis for this belief. The description given by Fourie of the skin lesions in the congenital porphyria of cattle leaves little doubt that these lesions were due to photosensitization, but it is to be hoped that use will be made of the cattle remaining at the Onderstepoort Veterinary Laboratory for action-spectra studies or other experiments on this aspect of the disease.

#### DISEASES OF TYPE III

Included in this group are most of the economically important livestock diseases in which photosensitivity has been reported. The criterion adopted for classification under this type is the occurrence of bilirubinemia (generally indicated by clinical icterus) or liver lesions, since study of the causal relation between hepatic damage and photosensitivity, or proof that phylloerythrin is the photodynamic agent, has been attempted in very few cases. In two diseases (Mathews, 1938; Steyn, 1943) it has been suggested that a pigment other than phylloerythrin is responsible, but in neither case is the evidence convincing. Although this possibility is admitted, the assumption that photosensitivity associated with liver dysfunction is due to deranged phylloerythrin excretion is considered justifiable, where evidence to the contrary is lacking, provided that the animal is consuming chlorophyll in its diet. A plea is entered at this stage for the examination of the blood for phylloerythrin and bile pigments in all investigations of photosensitivity in farm animals. Since the photodynamic aspects are similar throughout, the following discussion deals with only a few examples of this type selected to illustrate the general features of these diseases. Details such as the nature of liver lesions and the influence of climate and management on these diseases do not fall within the scope of this review.

*Geeldikkop (Yellow Thick Head).* This disease of sheep in South Africa was the first of the hepatogenous type to be carefully studied and may be taken as representative of this class. It is characterized by severe photosensitivity, icterus, and liver damage in sheep grazing the plant *Tribulus*

*terrestris* under certain conditions of growth. In attempts to reproduce various aspects of this disease, Quin (1933a) obstructed the bile flow of sheep by ligation of the common bile duct and thereby produced not only icterus but also photosensitivity. Rimington and Quin (1934) were able to identify in the blood of these sheep the pigment phylloerythrin, which was known to occur in the bile of herbivores. They were next able to demonstrate phylloerythrin spectroscopically in the blood of field cases of geeldikkop, and following the demonstration that phylloerythrin injected intravenously produced photosensitivity in sheep, they concluded that this pigment was the photodynamic agent in geeldikkop.

Phylloerythrin is a product of degradation of chlorophyll in the digestive tract of herbivores. Normally some phylloerythrin is absorbed into the portal circulation, but it is efficiently excreted by the liver into the bile. It is apparent that any damage to the liver which results in biliary obstruction or otherwise interferes with the mechanism of phylloerythrin excretion is likely to lead to photosensitization in herbivores, provided that they are at the time consuming food containing sufficient chlorophyll.

The substance responsible for this liver dysfunction of geeldikkop has not yet been isolated from *T. terrestris*. However, another plant, *Lippia rehmanni*, was shown by Quin (1933b) to produce effects similar to those in geeldikkop, and from this plant Rimington and Quin (1935) isolated the toxic principle, which they named "icterogenin." Later this principle was resolved into three isomers (Rimington and Quin, 1937a). From what has been determined of their structure and properties, these substances appear to be of the nature of resenic acids.<sup>1</sup>

The work of Quin and Rimington on geeldikkop and *Lippia* poisoning has been followed by the recognition that a number of other photosensitivity diseases of livestock, some of them causing considerable economic losses, show a similar etiology. The variety of poisonous plants and other conditions that may lead to hepatogenous photosensitivity is demonstrated by the list at the end of this section. In all cases the photodynamic aspects of these diseases appear to be identical—although in comparatively few have attempts been made to confirm the presence of phylloerythrin in the blood—but both the severity and type of the liver lesions and the nature of the hepatotoxins show considerable diversity. In some plants the toxin appears to be present always; in others it appears fleetingly only under certain conditions of growth. Some plants are toxic to most species of animals; others appear to affect ruminants only. Such factors greatly complicate attempts to isolate the hepatotoxins responsible for diseases such as facial eczema and geeldikkop.

*Congenital Photosensitivity in Southdown Sheep.* Unique among the hepatogenous photosensitivity diseases is that which occurs in certain

<sup>1</sup> For subsequent studies on the structure of icterogenin see D. H. R. Barton and P. de Mayo, J. Chem. Soc., 1954: 887-900.

lines of purebred Southdown sheep in New Zealand. Lambs that are normal while subsisting solely on milk become severely photosensitive when they begin to eat grass. There is no sign of structural damage or defect in the liver (Cunningham *et al.*, 1942), but slight bilirubinemia may be seen, and the rose-bengale excretion test also indicates defective liver function (Clare, 1945). The photosensitizing agent has been shown to be phylloerythrin (*ibid.*). Coproporphyrin I in small amounts was also identified in the blood, but it was established that this porphyrin does not contribute to the photosensitivity. Hancock (1950) has shown that expression of the disease is inherited as a Mendelian recessive.

This disease is of particular interest because it indicates that photosensitization by phylloerythrin may occur as a result of a more or less specific functional derangement that is not associated with structural changes and not betrayed by obvious clinical signs. It is possible that among photosensitivity diseases of uncertain etiology there may be similar instances of permanent or sporadic impairment of the phylloerythrin-excreting mechanism not accompanied by demonstrable liver dysfunction.

*Poisoning by Holocalyx glaziovii.* Photosensitivity in this disease of cattle in Brazil has been attributed by Rocha e Silva (1940) to the presence of a cyanogenetic substance in the plant. Arguing from his observations that cyanides increase the uptake of oxygen in photodynamic hemolysis (referred to earlier in this chapter), Rocha e Silva considered that hydrogen cyanide in the blood will so enhance the activity of phylloerythrin that small amounts present in the blood of normal animals will bring about photodynamic action. There appears to be little evidence to support this ingenious hypothesis, and even the nature and extent of the effect of cyanides on photodynamic hemolysis are still doubtful. Rocha e Silva gave cyanide in sublethal amounts over long periods to one animal and claimed that photosensitivity was produced, but his description of the symptoms scarcely justifies this conclusion. Photosensitivity is not seen in other examples of poisoning by cyanogenetic plants and did not occur in sheep dosed daily with sublethal amounts of potassium cyanide, although these animals were kept outdoors and received green feed (Van der Walt, 1944). The occurrence of phylloerythrin in the blood of normal ruminants has never been established, but, if present, it is there in very small amounts, since it has not been found in tests made in the writer's laboratory (D. D. Perrin, unpublished observations), although the method used would detect 0.003 mg per 100 ml. Furthermore sheep can be photosensitized during poisoning by *Panicum miliaceum*, a condition in which phylloerythrin is the photosensitizing agent (Clare, unpublished observations), before this detectable level is attained in the blood.

Some of the toxicity of *Holocalyx* appears to be due to a cyanogenetic substance, but as the reports by Rocha e Silva definitely indicate that

*Holocalyx* produced liver dysfunction, including icterus in the later stages, it seems most likely that the photosensitization is due to a derangement of phylloerythrin excretion. That photosensitization through such a derangement can precede both bilirubinemia (as indicated by the van den Bergh test) and macroscopic liver damage has also been established in work on the toxicity of *P. miliaceum* (Clare and J. E. V. Simpson, unpublished observations).

*Poisoning by Agave lechuguilla.*—Mathews (1937) reported that this disease of sheep and cattle involves both liver damage due to a toxic saponin and photosensitivity by a pigment absorbed directly from the plant. The evidence for this pigment being involved is strong, since extracts in which the saponin had been inactivated in various ways produced photosensitivity but no liver lesions. However, in investigations on another disease, produced by *Nolina texana*, in which apparently identical liver lesions are seen, Mathews (1940) again produced photosensitivity in rats without liver damage but this time considered that the photodynamic agent was phylloerythrin, because photosensitivity occurred only when chlorophyll was included in the diet. From this experiment it seems possible that both *A. lechuguilla* and *N. texana* may contain a substance that interferes with phylloerythrin excretion in addition to that responsible for the observable hepatic lesions.

This discussion of the work on *Holocalyx glaziovii*, *A. lechuguilla*, and *N. texana* emphasizes the desirability of carrying out tests for phylloerythrin in diseases in which photosensitivity is associated with hepatic lesions.

*Photosensitivity Following Injection of Phenanthridinium.* Photosensitization associated with liver damage and icterus has been observed in cattle injected with phenanthridinium 1553 during attempts to control trypanosomiasis (Bell, 1945, 1947; Stewart, 1947; Evans, 1948). No attempt seems to have been made to determine whether phylloerythrin is the photodynamic agent, but the reports indicate that it is. The toxic effects of the drug are influenced by factors not yet properly defined, and thousands of animals have been treated without ill effects—a fact that suggests that phenanthridinium itself is not the photodynamic agent. An unusual feature is the long delay—up to 6 weeks—between injection and onset of photosensitivity.

The rest of the diseases involving hepatogenous photosensitivity in animals do not require special consideration of the photodynamic agent, and therefore only brief reference is made to them here. It should not be inferred that they are of minor importance, for some, such as facial eczema and *Tetradymia* poisoning, have been responsible for serious losses of livestock.

The following list summarizes the diseases and pathological conditions in which photosensitivity has been observed as an accompaniment of liver dysfunction:

Bile-duct ligation: Phylloerythrinemia established (Quin, 1933a; Rimington and Quin, 1934).

Blockage of bile duct by cyst (Graham and Gordon, 1937).

Geeldikkop: Poisoning by *Tribulus terrestris*. Phylloerythrinemia established (Rimington and Quin, 1934).

Facial eczema: Occurs on mixed pastures during autumn in New Zealand (Cunningham *et al.*, 1942). Phylloerythrinemia established (Clare, 1944). Ether extracts of toxic grass produce lesions in guinea pigs (Perrin *et al.*, 1953).

*Lippia* poisoning: *L. rehmanni* Pears. Toxic principles are of nature of resenic acids; named "icterogenins" (Rimington and Quin, 1937a).

*Lantana* poisoning: *L. camara*. Toxic principles resemble resenic acids; named "lantadenes" (Louw, 1943, 1949).

*Lechuguilla* poisoning: *Agave lechuguilla*. Plant contains a saponin causing liver and kidney lesions, but a photosensitizing pigment may be present in the plant (Mathews, 1937).

Sacahuiste poisoning: *Nolina texana* (Mathews, 1940).

Ngaio poisoning: *Myoporum laetum*. Toxic principle is in the essential oil (Cunningham and Hopkirk, 1945). Phylloerythrinemia established (Clare, unpublished experiments).

*Panicum* poisoning: *P. coloratum* and *P. laevifolium* (Rimington and Quin, 1937b). *P. effusum* and *P. decompositum* (Hurst, 1942). *P. miliaceum* (Rottgardt, 1944). Phylloerythrinemia with *P. miliaceum* established (Clare, unpublished observations).

Alecrim poisoning: *Holocalyx glaziovii* (Rocha e Silva, 1940).

Waterbloom (algae) poisoning: *Microcystis flos-aquae* (Brandenburg and Shigley, 1947). *M. toxica* Stephens (Steyn, 1943). Liver toxin is an alkaloid (Louw, 1950).

Yellowses: Occurs on mixed pasture in Scotland (Greig, 1943).

*Tetradymia* poisoning: *T. glabrata* (Clawson and Huffman, 1937). Petroleum-ether extracts toxic (Fleming *et al.*, 1922).

Black fever: *Kochia scoparium* (Rottgardt, 1944).

Lupin poisoning: *Lupinus angustifolius* (Brash, 1943).

Phenanthridinium injection (Bell, 1947).

Congenital photosensitivity in Southdown sheep: Phylloerythrinemia established (Clare, 1945).

#### PHOTOSENSITIVITY OF UNCERTAIN ETIOLOGY

There have been numerous reports of photosensitization, often associated with specific plants, which cannot be definitely assigned to either the primary or hepatogenous types on the meager information available. It is probable that most of these diseases belong to the primary type, since obvious signs of liver damage have not been reported, but it is possible that some of them are due to a transient specific derangement of

phylloerythrin excretion. Chief among these diseases of uncertain etiology are trefoil dermatitis, associated with various species of *Trifolium* and *Medicago* species (Hurst, 1942); rape scald, seen occasionally in sheep grazing on *Brassica rapa* (Cunningham *et al.*, 1942); and *Erodium* photosensitivity, caused by *Erodium cicutarium* (Hurst, 1942), and *E. moschatum* (Clare, unpublished observations).

#### PHOTODYNAMIC ACTION IN HUMAN AILMENTS

It is necessary to preface the following references to human diseases with a reminder that photodynamic action is not synonymous with photosensitivity. The triple response to blue and violet light (Blum, 1941a) and the sensitivity shown by some persons after sulfanilamide therapy (Blum, 1941b) are two examples which have been shown to be independent of molecular oxygen and which therefore do not fall within the definition of photodynamic action. (It is quite possible that similar sensitivities occur in lower animals also, but such rare reactions of individuals would be less noticeable among animals and not likely to be the subject of specialized investigation.)

Those reactions of human beings to light which do appear to be due to photodynamic action are generally of the type defined as primary in the section on animal diseases. The one possible example of Type II is the photoreaction associated with certain forms of porphyria, but, as will be indicated later, the etiology of these lesions is extremely doubtful. There is no evidence of hepatogenous photosensitivity occurring in human beings, and since the chlorophyll content of the human diet is comparatively low and the conditions of the human digestive tract are not so favorable to phylloerythrin formation as those in the ruminant stomach, this type of photosensitivity is scarcely to be expected.

*Photosensitivity Following Medication with Dyes.* According to Blum (1941a), accidental photosensitization has resulted from therapeutic use of eosin (in epilepsy), tryptoflavine (for gonorrhoea), and hematoporphyrin (for melancholia) and after injection of rose bengale as a test for liver function.

*Photosensitivity Due to Contact.* There have been a number of reports of photosensitivity through contact of the skin with fluorescent substances or materials containing them. Blum (1941a) cites instances attributed to contact with grass, figs, parsnip, perfumes containing bergamot or citron oils, green soap, and coal-tar and petroleum derivatives. Most of these materials contain fluorescent substances such as chlorophyll, coumarin derivatives, and acridine compounds which might have produced the effects provided conditions were favorable for penetration through the corneum. The need for light to elicit the effects has been demonstrated in some cases, and definition of action spectra has been

attempted. Kuske (1940) found that extracts of various plants, including parsnip and fig, produced sensitivity to light of wave lengths between 3340 and 3660 Å, with some effect at wave lengths extending up to 4120 Å, and concluded that the photodynamic agent was fucocoumarin and related substances. Foerster and Schwartz (1939) produced sensitivity to light in the range 3900-5000 Å by application of pitch and coal tar and considered that acridine and anthracene are not the only photosensitizing agents in pitch dermatitis.

Photosensitivity in orchard workers spraying suspensions of phenothiazine has been reported (De Eds *et al.*, 1940). Experiments carried out by De Eds *et al.* suggest, however, that this sensitivity was due not to contact of the phenothiazine with the skin but to oral absorption of the spray, leading to production of thionol, which then reached the skin via the blood stream.

*Photosensitivity after Treatment with Sulfanilamide.* It is well recognized that some persons develop sensitivity to sunlight after treatment with sulfanilamide and allied substances. The report by Rimington and Hemmings (1938) that an increased formation of coproporphyrin also accompanied the use of these drugs encouraged the belief that this light sensitivity was due to photodynamic action by porphyrins. Blum (1941b) found that the radiation to which human skin was sensitized after injection of sulfanilamide was restricted to wave lengths below 3200 Å and that molecular oxygen was not needed to evoke this response. Furthermore this response was not immediate, thus resembling sunburn rather than photodynamic action. Blum concluded that photosensitivity due to sulfanilamide is not an example of photodynamic action but probably represents an increased sensitivity of the sunburn mechanism of normal skin.

*Association of Photosensitivity with the Porphyrrias and Hydroas.* The occurrence of hydroa or hydroa-like lesions in cases of congenital porphyria is still freely interpreted as porphyrin photosensitization in view of the established photodynamic activity of these compounds; yet as early as 1923 Garrod clearly indicated that the association of hydroa and porphyria could not be explained simply in this way. The effects of exposure to light after injection of porphyrins are of a different nature from those occurring in the hydroas; even in hydroa patients it has been difficult to produce the typical eruption by irradiation. The wave lengths that have produced effects in successful experiments do not agree with the absorption spectra of porphyrins; hydroa lesions may occur without elevated porphyrin excretion; some forms of porphyria, such as acute idiopathic porphyria, are not accompanied by hydroa; and in some cases of hydroa bullous lesions are produced by slight trauma without exposure to light. Garrod concluded, however, that sensitivity to light resulting from the presence of porphyrins in the tissues must be the cause of the

hydroa and presumed that faulty technique was responsible for failure to reproduce the natural syndrome.

Numerous attempts have been made to resolve some of these apparent inconsistencies, but none has proved satisfactory. For example, explanations based on the occurrence of the porphyrins in the tissues as colorless precursors, on protection by pigments such as urofuscin, or on the variation of the photosensitizing power of different porphyrins all fail to account for the difference between hydroa lesions and those resulting from photosensitivity.

The relation of porphyrin metabolism to diseases of the skin was reviewed by Turner and Obermayer (1938) and by Brunsting *et al.* (1939), and the general conclusion from both reviews is that there has been no clear demonstration that porphyrin photosensitization is directly responsible for the skin lesions seen in diseases involving a derangement of porphyrin metabolism. Essentially the same conclusion was drawn by Blum (1941a) in a comprehensive discussion of attempts that have been made to reproduce these lesions experimentally and to determine action spectra.

Blum and Pace (1937) could not elicit typical symptoms in a patient with hydroa vacciniforme by exposure to sunlight or to artificial sources of radiation. In the course of these experiments, however, one of the normal subjects, in whom local reactions had been produced by injection of hematoporphyrin and repeated exposure to sunlight, developed papulovesicles 9 weeks later at the site of injection. Blum (1941a) suggested that these lesions may be similar to those obtained by Meineri (1931), who found that successive exposures of a hydroa patient to light did not give lesions directly but made the skin more sensitive to slight trauma. Blum considered that Meineri's explanation of the development of hydroa-like lesions is the one most consistent with the known evidence and justifies further study. Whether such increased sensitivity to trauma can be a delayed result of photodynamic action when other reactions are not elicited has yet to be determined.

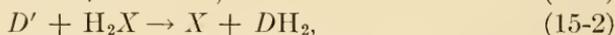
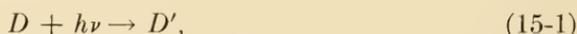
The whole subject of the relation of endogenous porphyrins to photosensitivity diseases remains in an unsatisfactory state. Although the ability of certain porphyrins (more especially the artifact hematoporphyrin) to produce photosensitivity is well established, there appears to have been no clear demonstration of photodynamic action by porphyrins in any of the diseases of man. Too frequently investigators of photosensitivity in such diseases have made no attempt to determine the nature, isomeric type, and amount of the porphyrins in the urine and blood of their patient—observations that may influence future interpretation of their work—and those interested in porphyrin metabolism have often neglected the photosensitivity. This subject well illustrates the need, in all work on photodynamic action, for careful experiments along

the lines indicated in Blum's three postulates, together with a recognition of the differences between sensitivity to light and photodynamic action.

#### ADDENDUM

Since this chapter was completed, several papers dealing with the mechanism of photodynamic action have reached the writer. *In vitro* experiments (Galston, 1950; Weil and Buchert, 1951) indicate that histidine, tryptophan, and tyrosine are the groups in proteins most susceptible to photosensitized oxidation, whereas cystine and methionine are little affected. However, Calcutt (1951) suggested that irradiation makes sulfhydryl groups more vulnerable to oxidation and attributed to this the hastening of the photosensitized killing of paramecia that had been irradiated before addition of the photosensitizer.

Galston (1950) has shown that the growth of sections of etiolated pea epicotyl is inhibited by light in the presence of riboflavin through photosensitized oxidation of indoleacetic acid, and that in the plant itself a similar action occurs with a flavoprotein as the sensitizer. In the mechanism postulated for this reaction, the light-activated riboflavin acts as a hydrogen carrier between the indoleacetic acid and oxygen. Weil and Maher (1950) propose a similar mechanism for photosensitized oxidation of nicotine by methylene blue, and since they detected hydrogen peroxide during the course of the reaction, Weil and Maher attribute the final stage to oxidation by peroxide. Furthermore the role of the light-activated flavoprotein enzyme of Galston is the production of hydrogen peroxide, which through a peroxidase oxidizes indoleacetic acid. The photodynamic aspects of these reactions may be expressed in the following terms:



Here  $D$  is the sensitizer, and  $H_2X$  is a hydrogen-donor substrate. Thus, in the system studied by Galston,  $D$  is the flavoprotein,  $H_2X$  is indoleacetic acid, and the final stage (not shown in the equations) is the oxidation of the indoleacetic acid by peroxide-peroxidase; in that of Weil and Maher,  $H_2X$  is nicotine,  $D$  is methylene blue, and in the final reaction the product  $X$  of Eq. (15-2) undergoes further oxidation by peroxide. In both systems carbon dioxide is an end product of the series of reactions.

The mechanisms suggested in these reactions emphasize the possibility that photodynamic action may proceed by various paths according to the circumstances. There is a need for critical study of these various mechanisms using sensitizers of various classes and with measurement of oxygen consumption, substrate disappearance, and ideally of the nature

and amount of intermediates and reaction products, including tests for hydrogen peroxide.

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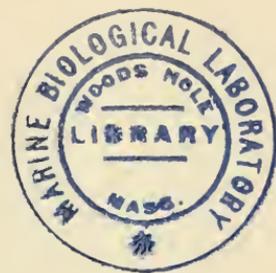
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