

Electronic Properties of Natural and Modeled Bilayer Membranes

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INTRODUCTION

Natural membranes are characterized by high selectivity, specificity, and control processes whose mechanisms of operation can be reduced to molecular levels. Many of these processes involve self-assembly of macromolecules such as lipids, proteins, and DNA. In 1941 Szent-Gyorgyi suggested that proteins and other materials may possess some of the solid-state electronic properties known to occur in amorphous materials and organic polymers (1). Since then, electronic processes in living systems have been extensively studied, especially in connection with coupled redox reactions in mitochondria, energy transfer and conversion in chloroplasts, and sensory transduction in visual systems. Bilayer lipid membrane-based structures responsible for carrying out the aforementioned processes may be the transducers. Whether we can construct similar membrane devices by emulating natural systems remains to be seen, but the approach is a viable one. The purpose of this chapter is to review data and concepts involving the study of electronic properties of both natural membranes and experimental lipid bilayer systems such as planar bilayer lipid membranes (BLMs) and spherical liposomes. Although the emphasis will be on the BLM systems, it is appropriate to deal first with natural membranes and then proceed to their models because the study of BLMs originated from attempts to better understand processes in natural membranes. Jointly studying natural membranes and artificial lipid bilayers, leads to both a better understanding of natural membranes, and the possibility of constructing bilayer systems capable of performing basic biological functions such as photosynthesis (2-5).

Planar and spherical bilayer lipid membranes can be made from constituents of natural membranes, combinations of natural membranes and artificially synthesized components, or completely synthetic components (6).

The problem of electronic conductivity in both biological membranes and experimental lipid bilayers has a diverse background. One related area involves the study of the semiconductive properties of basic classes of chemical compounds of living

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organisms, paralleled by the theoretical study of the quantum chemistry of biomolecules. Another area involves attempts to link knowledge of physiological functions with electronic models of living processes (7-10). Neither of these related fields will be dealt with here, but they are treated elsewhere (1, 11-14). Results of investigations of electronic properties of other models of biological membranes such as thick lipid films and multi-lamellar layers will also not be dealt with here. BLMs and liposomes have proven to be, in many respects, the most adequate models of biological membranes.

ELECTRONIC PHENOMENA IN MEMBRANES

Electrical current may arise as a result of movement of any kind of charged species, or from a change of polarization. In biological materials, currents of ions, electrons, and polarization phenomena are so highly interrelated that it is difficult to separate the individual components. But special techniques to do so have recently become available. Moreover, there is a group of specific phenomena that may be adequately explained only in terms of electron transfer and redox reactions.

MOLECULAR AND SUPRAMOLECULAR ASPECTS OF ELECTRON TRANSLOCATION

Every reaction in which transfer of an electron occurs from a reductant species, R, to an oxidant one, O, is called a redox reaction. However, the physical conditions under which the transfer occurs and the mechanisms of the electron transfer may vary. The factors that determine the mechanism of electron translocation are the ability of one molecule to donate or to accept an electron (redox potential), the geometry of the system in which the molecules and/or atoms are located (liquid or liquid crystal phase, solid crystal-like or noncrystalline phase), and various externally changeable factors such as temperature, pressure, and illumination. Redox reactions may be divided into five subgroups: (1) redox reactions occurring through collisions in solution or at a solution/solid interface; (2) charge-transfer reactions in which the electrons are shared between the components of the charge-transfer complex; (3) translocation of electrons in a solid phase where electrons overcome the relatively low energy barriers between molecules or atoms by activated hopping over a barrier; (4) quantum-mechanical tunneling of electrons between sites separated by a relatively high-energy barrier; (5) conduction of electrons along common energy bands. Taking into account the physical situation of biological membranes, one may confine the first two mechanisms to the solution/membrane interface, and the last three mechanisms to the membrane itself.

1. Collision Redox Reactions

The transfer of whole numbers of electrons takes place according to the scheme:



When R and O are reductant and oxidant species, respectively, R^+ is the oxidized reductant and O^- is the reduced oxidant. The result of these redox reactions depends on the redox potential difference between species, and the frequency of collisions determined by the temperature, and the concentration of both chemicals. The redox potential (or oxidative ability) of molecules of biological significance has been given (15). Equation (1) may be expressed in terms of electron acceptor (A) and donor (D). In this case we have reduced acceptor (A^-) and oxidized donor (D^+).

2. Charge-Transfer Reactions

If two chemical species form a charge complex, they share an electron in a common orbital. The probability of finding the electron either on the donor side or the acceptor side may differ, depending mainly on the difference between the ionization potential of the donor and the electron affinity of the acceptor. In the excited state of the complex, the shifting of the electron is much stronger. When an electron is strongly shifted towards the acceptor side, dissociation of the charge-transfer complex occurs, and the result may be regarded as a redox reaction.

3. Hopping

This occurs when two electron energy states exist that are isolated from each other by an energy barrier. Commonly, the mobility of the electrons and the conductivity of the material (a solid) are both low. However, if the height of the barrier is lower than the energy gap between the valence and conduction bands, activation of electrons by either an electric field or temperature will increase charge-carrier mobility, but not density.

4. Tunneling

The quantum-mechanical probability of tunneling across an energy barrier depends on its thickness and shape, and on the energy and mass of the particle. In biological systems, tunneling may be a mechanism of electron translocation over distances of tens of Angstroms (16). Tunneling of electrons in biocompounds was discovered by Chance, DeVault, and their co-workers (17, 18). The probability of tunneling may be altered by variations in temperature or by applying an electric field, because these factors influence the shape and width of the energy barrier as well as the density of electrons and their effective masses. The macroscopic consequence of these changes are reflected in the conductivity of the material.

5. Conduction Along Energy Bands

In inorganic crystalline materials, common energy bands arise as a result of

periodicity and overlapping of wave functions of electrons of a great number of atoms forming the solid. However, biological material is not periodic and homogeneous, therefore the formation of extensive common-energy bands is doubtful. Yet, one may expect the formation of localized common-energy bands which may be generated as a result of aggregation of a sufficient number of molecules having π -type bonds. The width of the conduction band (and thereby the effective mass of the charge carriers moving along them) is determined by the degree of overlapping of the π -electron systems of the molecules. The value of the forbidden energy gap, which is the crucial factor that determines the number of the charge carriers in the conduction band, depends primarily on the electronic structure of the atoms forming the given material, and on their proximity and periodicity. The common energy bands may extend through the whole solid or occur only locally, being isolated from other similar regions by energy barriers.

Temperature is the main factor governing the population of electrons that are free to move in the conduction band. The conductivity span of materials classified as semiconductors is 10^{-10} – $10^1 (\Omega m)^{-1}$. However, the magnitude of the conductivity does not uniquely determine either the nature of the charge carriers or the mechanisms underlying the conductivity. Many ionic conductors and even insulators also have conductivities falling into this range. Therefore, a specific set of criteria is usually applied to identify the electronic nature of the semiconductivity of a material.

The conductivity of a semiconductive material should vary with temperature according to the formula:

$$\sigma(T) = \sigma_0 \exp(-E_g/2kT) \quad (2)$$

where $\sigma(T)$ is the value of conductivity at a given temperature, σ_0 is a constant (dependent primarily on the mobility and effective masses of the carriers), E_g is the energy gap separating the valence and conduction bands, k is the Boltzmann constant, and T is the absolute temperature. The value of E_g is usually given in eV; in semiconductors, it is 1–3 eV.

Intrinsic semiconductors are particularly sensitive to the presence of doping substances, which can change the value and character of the conductivity (holes or electrons). An especially important feature of the investigations on extracted biological materials is that they contain impurities which substantially influence the conductivity. The presence of ions and water (which decrease E_g by increasing the dielectric constant of the material, and act as electron donors) adds to the complications in characterizing the nature of the semiconductivity of biological materials (19). Another characteristic of some semiconductive materials (those in which E_g is lower than the energy of impinging light quanta) is the rise in conductivity that they exhibit upon illumination. This property is manifested in the photovoltaic effect. When one type of current carriers predominates,

the change of conductivity upon illumination of the material is given by:

$$\Delta\sigma = e(u_+ \Delta C_+ + u_- \Delta C_-) \quad (3)$$

where $\Delta\sigma$ is the change of conductivity, e is the elementary charge, u_+ , u_- are the hole and electron drift mobilities respectively, and ΔC_+ , ΔC_- are the increases in concentration of the holes and electrons, respectively. The photovoltage generated in open circuit conditions, E_{op} , is given by:

$$E_{op} = (kT/e) \ln(1 + \sigma_l/\sigma_d) \quad (4)$$

where σ_l and σ_d are the conductivities in light and in dark, respectively.

The thermoelectric effect is a phenomenon that occurs at a junction between two materials having different electrical and thermal conductivities, and different temperatures, T_1 and T_2 . In this system a voltage, E_{11} , is generated that depends on the temperature difference and the relative thermoelectric power, E_{12} (defined as a voltage generated across the junction when the temperature changes by one degree). E_{12} has a positive value when the current generated flows from the conductor 2 to the conductor 1.

$$E_{11} = \int_{T_1}^{T_2} E_{12} dT \quad (5)$$

The thermopower generated in systems containing semiconductors is a very sensitive function of the level of impurities present. The magnitude of E_{12} in systems based on contact of a semiconductor and a metal usually exceeds by more than an order of magnitude that found in systems based on metal/metal contacts.

The presence of a junction between p-type and n-type conducting materials also gives rise to some characteristic conductivity phenomena. One of them is rectifying action and its derivative—Zener-diode behavior. In a rectifier, the current has a much smaller resistivity in one direction than in the other, and in the Zener-diode, after the voltage across the junction reaches a certain value, it maintains a steady level.

Thus, all the above possible mechanisms of the translocation of the electrons create a spectrum extending from the most simple mechanism (collisions), through relay mechanisms to those involving large aggregates of atoms or molecules. (Figure 1). In a broad meaning of the term “redox reactions,” all of these mechanisms may give rise to interfacial, intramembrane, and transmembrane processes of reduction and oxidation. In both natural and artificial lipid bilayers immersed in an aqueous solution, it is possible that many of these mechanisms are operating at the same instant, with one of them being

the prevailing or limiting factor.

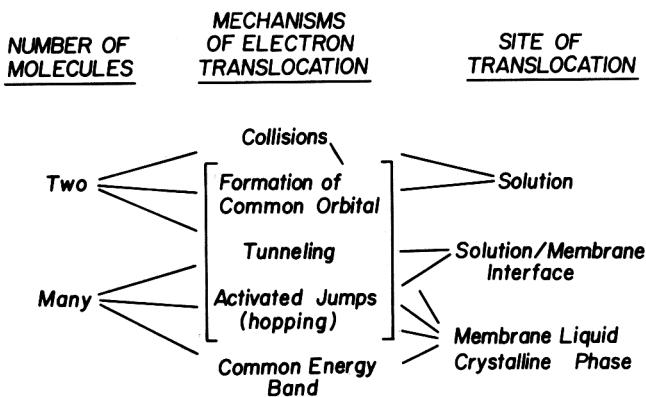


Figure 1. Possible mechanisms of electron translocations in natural and artificial bilayer lipid membranes. The bracketed mechanisms, when operating on molecules or atoms, may give rise to long-distance electron translocations. The conduction brought about by the existence of donor or acceptor levels located near the conduction or the valence band respectively, may be treated as intermediate between the hopping and band mechanisms. Thermally or electrically activated injection of electrons from the donor levels into the conduction band, or creation of holes in the valence band by the presence of acceptor levels, constitute the current flowing in these bands.

ORIGIN OF CONDUCTIVITY

Ionic currents undoubtedly play an important role in both natural and artificial bilayer lipid membranes. However, experimental evidence of electronic conductivity in materials extracted from biological structures, as well as information gathered on the biochemical and biophysical processes taking place in the course of physiological activity of living membranes, lend support to the idea that electrons and/or holes must also play an important role in life processes. In this connection, a question arises of how to distinguish between electronic and ionic conductivity either in natural or artificial membranes. In principle, it is possible to tell the difference using methods that allow detection based on any of the following: (1) differences in mass; (2) structure of the material determining the behavior of the carrier; (3) energy requirements for initiating the conduction process; (4) specific reactions at the interface (electrostenolysis) (19).

1. Mass of Charge Carriers

An electron is nearly 2000 times lighter than a proton and much lighter than simple ions or molecular ions, and this difference manifests itself in many ways. The mobilities of electrons in solids are higher than those of ions, but the existence of energy barriers and the narrowness of the conduction bands (high effective masses) decrease the mobilities of electrons and holes. High-frequency techniques made it possible to overcome difficulties arising from the existence of energy barriers at the boundaries of

microstructures in biological materials.

Another difference arising from that inequality of masses of ions and electrons is the time required for the development of a voltage or current response to an external factor such as a light pulse.

If a magnetic field is used to induce circular motion of electrons around the magnetic field lines, two kinds of reactions to the field may be observed: (1) voltage development is slower in the case of ions than electrons; (2) the cyclotron frequency of electrons is higher than that of ions.

When charge translocation occurs, the increase in weight of the part of the system where the ionic carriers are deposited may be observed. With the transfer of ions will be a concomitant polarization arising from the ions deposited in that part of the system.

2. Structure of the Material

A generally accepted structure of a biomembrane is depicted in Figure 2. One unique feature to be noted, as a result of the ultra thinness of the structure, is the field effect. If a potential as low as 10 mV exists across the membrane, electric field gradients on the order of 10^6 V/cm will be generated. If the membrane is asymmetrical, the current-voltage curve may be non-linear because of at least three mechanisms, each of which is analogous to well-known mechanisms in solid-state physics:

- 1) *Charge injection.* When an insulating layer is interposed between two conducting layers, an applied voltage will effectively be dropped across the insulating layer. Because of the interplay of thermal diffusion and the electric fields (electrical double-layer theory), the space-charge distribution is a function of the applied voltage. The result is that the greater the applied voltage, the higher the majority-carrier concentration and, therefore, the higher the charge injection.
- 2) *The image-force effect.* Image forces are an expression of the polarization of the dielectric environments of charged species. The forces exist near the membrane/electrolyte interface, and lead to a strong attraction of any charged species dissolved in the lipid bilayer. The effect is greater at higher voltages.
- 3) *The Wien effect* (20). The applied electric field affects the equilibrium constant of dissolved charged species, leading to a net increase in dissociation, and therefore to more charge carriers.

In well-organized materials such as membranes, liquid crystals, and thin films, it may be expected that the conductivity will be brought about by electrons or holes in the conduction or valence bands (5, 21, 22). Therefore, crystallographic studies of biological structures may give some hints regarding the expected nature of charge carriers. The

thermal behavior of the conductivity in such structures is described by Equation (2). To explore the electronic nature of the conductivity, additional tests may be carried out such as studying the effect on conductivity of infrared irradiation, and measuring the energy localization of traps by studying the time and temperature dependence of the delayed luminescence.

3. Energy Requirements

When transfer of charge occurs by ionic motion across a membrane where at least one very high energy barrier must be overcome, considerable activation energies of conductivity should be involved. According to the Born expression (if dielectric constant of the bathing solution is much higher than that of the membrane, ϵ_m):

$$E = q^2 / 2r\epsilon_m \quad (6)$$

where E is the energy spent overcoming the barrier at the interface, and q is the electronic charge on the ion. According to Equation (6), the energy necessary to transfer an ion of radius 10^{-10} m from water to a lipid medium is about 22.6 eV. Much less energy is needed to form a charge-transfer complex (0.02–0.54 eV per complex).

In tunneling processes it is not necessary that the energy be supplied externally, but external energy may change the height, shape or width of the barrier thereby altering the efficiency of the process.

For both intrinsic and extrinsic semiconductors, the conductivity will increase with increasing number of charge carriers in the conduction band (or holes in the valence band), and thereby increase their mobility. The factor determining these parameters is the temperature, and the energy expenditure necessary to bring about a certain value of conductivity in a given material may be estimated from Equation (2). However, in the case of biomembranes and BLMs, the conductivity may also vary with composition because of water dissociation (19).

4. Electrostenolytic Effect

This process involves reactions of oxidation and reduction at the opposite ends of an electronically conducting but high ion resistivity path (Figure 2). The process of the electrostenolysis across BLMs has been described previously (23). One of the most spectacular methods of demonstrating that electrons are transferred across the membrane is the formation of the highly reflective mirror, composed of reduced copper ions, at one of the BLM/bathing solution interface. Any reduction or oxidation taking part at the BLM/solution interface may be interpreted as electron conduction involvement in the process.

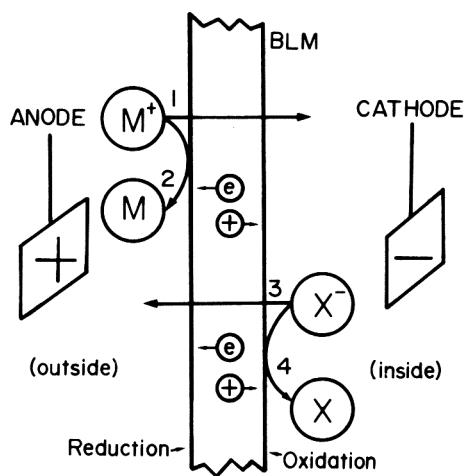


Figure 2. Mechanism of redox reactions across an ultrathin high-resistance membrane. BLM, bilayer lipid membrane; M^+ , metal ion or electron acceptor; M, reduced atom; X^- , anion or electron donor; X, oxidized atom; e, electron; +, hole. 1 and 3 denote ion conduction; 2 and 4 denote redox reactions (19).

ELECTRONIC PROPERTIES OF BIOLOGICAL MEMBRANES

INTRODUCTION

In reviewing experimental data, mainly investigations on whole cells will be considered. But since the process described will be primarily or entirely dependent on the cell membranes, they will be reviewed here.

Among other suggestions made by Szent-Gyorgyi in 1941, ushering in the solid-state physics approach to living processes, was that the cell membrane creates an outer boundary of the common energy levels in the cell (8, 9). This hypothesis has not been proved in its general formulation, however increasingly more data have been accumulated to support the notion that solid-state electronic processes can be ascribed to biomembranes. Strong support for the concept is provided by the results obtained in BLM research, which will be dealt with at length later.

Tunneling of electrons between membrane-bound, charge-transfer species and between molecules inside the membrane is one mechanism of electron transfer in biological membranes. It is considered an alternative mechanism to charge and energy transfer along the common energy bands (16-18).

The earliest suggestion that redox reactions may be the cause of transmembrane potential differences was made by Lund in 1928 (19, 24). However, the idea was discarded on the basis of the argument that there are no electronically conductive paths in biological membranes. A new momentum was given to the idea when it was discovered

that certain kinds of biologically significant compounds can be semiconductive. In 1962, Jahn renewed the hypothesis, suggesting that the electron-conductive paths across the membrane may consist of molecules that possess conjugate bond systems such as astacene, retinene, carotene, and vitamin A. The electromotive force moving electrons across the membrane was suggested to be due to differences in electron pressures in two electron systems placed across the membrane. The electron source was hypothesized to be a redox system in which the electron was released by action of ATP (20). Simultaneously with electron transport along the conjugate-bonded molecule, proton transport was assumed to take place along small diameter water pores. In Jahn's theory, both electronic and protonic flow across the membrane were considered to be coupled to the secretion of Cl^- .

MEMBRANES ACTIVE IN THE DARK

1. Secretory Cells and Erythrocytes

On the basis of the relationship between current flowing across the membranes of various secretory cells of many types of animals and the applied voltage, Mandel (25) concluded that the best approximation of the nature of processes taking place in the membrane is electron flow between a solution and semiconductive membrane. The hypothesis that electrons may be charge carriers passing through secretory cell membranes of the salivary gland of the Lone Star tick was tested by Pohl and Sauer (26). Using a suitable redox reaction indicator (Nyle Blue A), they were able to show that if a potential difference was created across the membranes (inside of the gland being negative) a color change from blue to red of the outside solution should take place. This result was interpreted as an indication of transfer of electrons across the membranes.

The flow of electrons across the erythrocyte membrane was shown in the following experiment by Marinov (27). Erythrocytes were isolated from the blood of rats, and after the hemoglobin inside them was converted into oxidized form (by incubation of the erythrocyte in the solution containing hydroxylamine), they were put into a solution containing eosin and NADH. After illumination of the suspension with visible light, the shift in the Soret band and the appearance of an absorption band with A_{max} at 570–580 nm, indicative of reduction of hemoglobin, was observed. Simultaneously, the shift of the Soret band of extracellular NADH–eosin complex, and the appearance of an absorption band in the region of 580 nm was seen. These redox reactions must have been coupled by the membrane of the erythrocyte. On the basis of additional measurements, the mediation of membrane mobile electron carriers was excluded. The only possibility which remained was that protein (spectrin) spanning across the membrane was able to transfer electrons from the donor to the acceptor located on opposite sides of the membrane.

2. Muscle

Semiconductive electronic processes may be manifested as increases in the conductivity, thermoelectric force or photocurrent generation. All of these effects were observed in muscle membrane. A temperature-related increase in conductivity was demonstrated in the frog sartorius muscle in the region 6–24°C (28). Above this temperature, up to 42°C, there were no changes in conductivity, and above 42°C the changes were exponentially related with the temperature as predicted by Equation (2). The calculated value of E_g was about 2 eV. No measurements aimed at a more specific determination of the nature of charge carriers (electrons/holes) were carried out. The differential thermoelectric power induced by the change in temperature was $230 + 40 \mu\text{V}/\text{deg}$ (29). Below 19°C the dominating charge carriers were positive, and above 23°C they were negative. The ability of the muscle to respond thermoelectrically was abolished by treating it with chloroform. In contrast to the experiments described earlier, the measurements were done along the muscle cell membrane.

In another series of experiments, the heart of the frog was used (30). Its beating was stopped by deleting K^+ from the bathing solution. If eosin (a light sensitizer) was added and the muscle was illuminated, the beating action reoccurred. Although a small (about 1°C) rise in temperature during illumination was observed, it apparently was not the reason for the effect observed. It was hypothesized that the light acted on the membranes of the muscle cells, exciting electrons in the eosin, which then migrated along the network of π bonds in the membrane. The same mechanism of changes of the level of excitation seen in the sartorius muscle of the frog was suggested. If the muscle membranes were stained with eosin and subsequently irradiated either with ultraviolet or visible light, the threshold of stimulation decreased (31).

3. Nerve

In 1956 Ernst (32) tried to establish links between the responses of nerve and semiconductors to various physical factors such as temperature and presence of impurities. He hypothesized that the frequency modulation of nerve impulses may be due to some specific current effect in the membrane. The fact that procaine and thioguanine can arrest the action potentials initiated by Pacinian corpuscles was explained as a result of the interaction between π electron systems of these compounds and the nerve membrane (33). In these terms, and including also a charge-transfer interaction, he explained the blocking of the action potentials by veratrine and novacaine. Moreover, on the basis of formal similarities between generation of high-frequency oscillation by the Gunn diode and frequency modulated nerve impulses, Ernst pointed out that impulse modulation in the nerve might be due to an electronic mechanism similar to that involved in the Gunn diode.

Photovoltage and photocurrent stained giant axons of *Sepia* (27). During his experiments, Chalazonitis took precautions to eliminate the infrared component of the incident light. The sign of the generated photovoltage (V) was dependent on the nature and concentration of the used pigment. The rate of photodepolarization one second after illumination was taken as a measure of the interaction between the neuron and the incident light. The rate was dependent on the intensity of the incident light of specific wavelength (I_λ), temperature (T), the membrane potential (E_r), the partial pressure of oxygen (pO_2), and the time lapse (t),

$$dV/dt = A \exp(kT) \quad (7)$$

where A and k are constants dependent on I_λ , T , E_r , and pO_2 . The rise of I_λ above a certain level led to the generation of an oscillatory firing pattern of the nerve. The surface density of the photocurrent flowing across the membrane was estimated as being of the order 60 mA/m².

Electron excitation and electron transfer phenomena were believed to be the crucial mechanisms involved. It was pointed out that after exciting the electrons in the dye-molecule aggregates, trapped or freely moving electrons or holes may appear in the membrane. These free electrons (and also protons), before they reached their final acceptors, contributed to current flow across the membrane and played the main role in depolarizing it. The photovoltage was thought to be generated at the illuminated donor-acceptor junction between photoactivated hemoprotein and carotene-protein molecules. Cope showed that the conductance changes found by Chalazonitis obeyed the Elovich kinetic equation, and this was additional supportive evidence for the idea that semiconductivity was involved in the photoresponses (14).

An influence of light on the spiking activity of stained (eosin, neutral red, and Bengal rose) nerve membranes from several species was found by Lakatos (29). Although the spiking activity was evoked by illumination in less than 50% of the stained nerves, no spiking was observed either in illuminated unstained nor in non-illuminated but stained neurons. Similarly, as in the sequence of events suggested by Chalazonitis, it was assumed that after excitation of the electrons in the dyes, migration of charge and energy along electron conductivity paths in the excited membrane occurred. The final event was the depolarization of the membrane.

Ultraviolet radiation (260, 280, 313 nm) and visible light (405 nm) were shown to be effective in eliciting the firing activity of unstained neurons (34). However, if the neurons were maintained

Generation of a thermoelectric voltage in the sciatic nerve of the frog has been reported (29-31). The thermoelectric coefficient was found to be 45 + 15 μ V/deg, and it decreased with time of preservation of the nerve and vanished completely after 9 days.

4. Mitochondria

Over 90% of all energy coupling in aerobic organisms involves redox reactions intimately associated with lipid membranes of mitochondria. At present, there is not one system for which the detailed mechanism of the electron-transfer and coupling process is known at a molecular level (35). Some transmembrane structure is required for a system that channels electrons in a directional manner whereby charge separation and proton movement are linked to the formation of high energy molecules such as ATP. Notwithstanding the uniqueness of the photosynthetic thylakoid membrane (discussed below), what is particularly striking is the similarity to other electron-transfer chains, such as that in mitochondria. The cristae membrane respiratory electron transport chain may have evolved from the photosynthetic system (Figure 3).

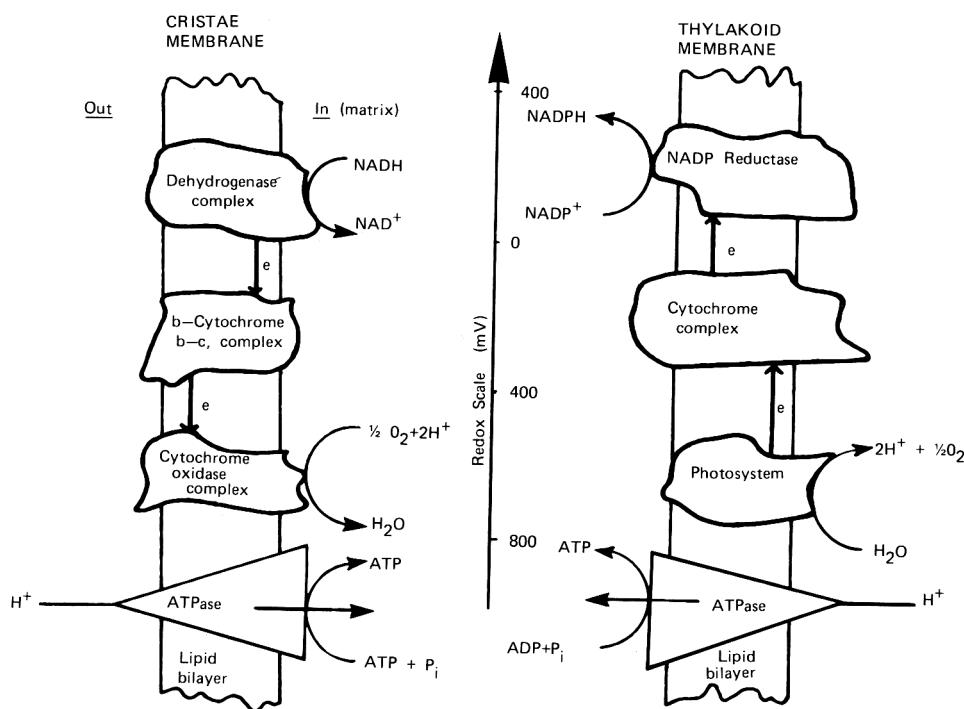


Figure 3. The bioenergetics of life processes in terms of the thylakoid membrane of the chloroplast and the cristae membrane of the mitochondrion. The energy-transducing membranes consist of two coupled redox reactions of photosynthesis and respiration, in which the products of one process are consumed by the other and vice versa (19).

In normal conditions, the flow of electrons is coupled to ATP formation which is known to be a universal biological energy carrier and storage unit. But before mitochondria were discovered and identified as the high-energy bond synthesizing apparatus in cells of aerobic organisms, Szent-Gyorgyi (8-10), referring to a previous suggestion made by Jordan in 1938, pointed out that energy transfer in cells may involve

a solid-state electronic mechanism. Specifically, Jordan hypothesized that in protein complexes insoluble in water, common energy levels of the outermost electrons may develop, and that an electron in such a band could move around in the band and drop to the ground level, releasing its energy in a place where it is in demand. This suggestion gave rise to an extensive study of electronic properties of biological molecules, both their semiconductive properties and their donor or acceptor abilities. Here, attention will be paid only to whole mitochondrial structures and their electronic properties.

Since it is difficult to investigate electronic processes in mitochondria performing their functions in an intact state, many simplified approaches have been developed. One of them is the theoretical investigation of the feasibility of tunneling between components of the respiratory chain, based on measurements of electron transfer chain components of simpler biological systems (16-18).

Free-charge mobilities in dried samples of mitochondria have been estimated (11, 12). The free charges were generated by a pulsed electron beam incident on a mitochondrial layer. Measurement of the speed of movement of the charges in an electric field interposed between two points on the layer allowed estimation of the electron mobility ($\mu \approx 5 \times 10^{-6} \text{ m}^2/\text{V} \cdot \text{S}$). If small quantities of water (less than 1%) were present in the sample, the value increased by one order of magnitude.

Using the Hall microwave mobility measurement technique, Eley et al. observed the movement of electronic charges in lipid extracts that contained components of the respiratory chain of rat liver mitochondria (1). The Hall mobility of electrons was about $5 \times 10^{-4} \text{ m}^2/\text{V} \cdot \text{S}$. If correction were made for the volumes involved, the mobility was calculated to be an order of magnitude higher. On the basis of thermoelectric force generation in pressed discs of a mixture of intact mitochondria and submitochondrial particles, it was shown that the majority charge carriers may be electrons (below the transition temperature, 342°K) or holes (above this temperature).

5. Plant Cells

Green plant photosynthesis is the process by which solar electromagnetic radiation is converted into chemical energy. This phenomenon is unique in that the light energy absorbed by pigment molecules embedded in a bilayer lipid membrane is converted eventually to stable energy-rich compounds such as ATP. The mechanism by which nature accomplishes this feat is not understood, and is being actively investigated by a number of approaches (36). Energy transduction by plant-cell membranes has broader significance beyond the process of photosynthesis itself. The mechanism of charge generation, separation and transport, and the coupling of separated charges to the production of ATP, is the central process of energy metabolism in all living systems. Thus, studies on algal cells and on pigmented lipid membranes, to be discussed in a later section, are of importance not only in their own right, but as a model for many similar

processes, such as those taking place in the cristae membrane of the mitochondrion (Figure 3).

As an accidental observation made during measurements of the impedance of simple algal cells, a decrease in the longitudinal resistance of about 50% was observed when the part of the cell on which the measurements were carried out was immersed in water (37). The possibility that these changes of conductivity might be due to an outward current flow from the cell was excluded. A hypothesis that the applied current was electronic was put forward, and experiments involving the measurement of Hall voltage were suggested. The Hall phenomena may be observed both in liquid and solid conductors and semiconductors. However, due to the low mobility of charge carriers in electrolytes and most organic semiconductors, the Hall voltage is very low. In experiments carried out on the internodal cell walls and membrane of *Nitellopsis obtusa* using magnetic fields of 0.8–2 T and applied transmembrane currents of 1.3–2.2 μ A, the Hall voltage ranged from 100–650 μ V. In dead cells, no Hall voltage was observed.

Changing the resting potential in plant cells under the influence of light is a well-known phenomenon (38, 39). However, these responses are not necessarily connected with the direct illumination of chloroplasts. The possibility that light may directly influence the resting potential of a plant cell membrane must be considered because this kind of interaction has been observed in membranes of rhizoids of some algae where only extremely small quantities of chlorophyll may be found (40).

To test the hypothesis that the light may also act directly upon algae membranes, ultraviolet radiation was used (37). After switching on the light, fast changes (much less than 1 sec.) of the membrane resting potential were recorded. The possibility that the observed changes may be due to UV absorption by lipids and proteins present in the membrane, and subsequent changes of its conductivity, was discussed and considered to have only minor importance.

MEMBRANES ACTIVE IN THE LIGHT

In spite of the minuteness of biologic photovoltaic converters, rod sacs and thylakoids, some data has been obtained using microelectrodes. In studying the electronic properties of both types of organelles, the following methods have proved useful: (1) study of the dependence of the conductivity on temperature; (2) study of the temperature dependence of the delayed luminescence; (3) high-frequency conductivity measurements; (4) measurements of photopotentials evoked in visual receptors and chloroplasts; (5) electrochromic shift measurements.

1. Visual Receptors

As in the case of the thylakoid membrane, to be considered in the next section, the

molecular mechanisms of light transduction by visual receptor membranes is obscure, but they appear to function as photon detectors, converting light quanta into electrical signals to trigger an action potential. How the absorption of light in the pigmented sac membrane is coupled to the electrical event in the plasma membrane is not known with certainty, although a hypothesis has been suggested (41). If photoreceptors are excited by a short flash, very fast photopotentials known as early receptor potentials (ERP) are generated. The ERP is usually biphasic, consisting of a corneal positive R_1 phase followed by a corneal negative phase. The R_2 of ERP's has been shown to be temperature dependent. Thus far, the ERP has not been detected in aqueous suspension of rhodopsin, or with aqueous suspension of rods and cones. This suggests that the generation of ERP requires the presence of pigments in an intact sac membrane, and has prompted a number of investigators to study dried rods and tissues. Some of the results obtained have been compared with the ERP of visual receptors and are reviewed in the following paragraph.

Trukhan and others (42, 43) have shown that dried rods of the visual receptor of the sheep obey Equation (2), which gives a calculated value 2.3 eV. The rods exhibited a photocurrent 2–3 times greater than the dark current. Action spectra of the rods were similar to those of rhodopsin. More sophisticated experiments, avoiding some difficulties arising from use of the steady current, were also carried on the dried pigment epithelium of the frog's eye. By using a high-frequency non-contact method it was possible to show that the photoconductivity rose with increasing light intensity, the photoconductivity and dark conductivity increased with water content of the sample (3–40%), and that the charge carriers were holes with a mobility on the order of $1.5 \times 10^{-3} \text{ m}^2/\text{V} \cdot \text{S}$. Protons were an alternative to the charge carriers responsible for the observed behavior of the sample (42).

Electron conductivity was taken into account as an alternative for apparent proton conductivity along the membranes of the outer segments of the frog's retina (44). In this experiment, the rods' admittance changes (15 Hz–60 KHz) were tested in various solutions, including some that had a water content close to normal.

The ERP was discovered in 1964 in monkey retina (45), and has been found in the pigments in the visual receptor in invertebrates and in vertebrates (45–47). It is believed that this kind of electrical response is a common feature of structures containing pigments.

The ERP consists of three phases, the last and slowest being identified with the α -wave of the electroretinogram. Both the fast-positive and the fast-negative phases have proved to be independent of ionic movements across the pigmented membrane on the basis of the following experimental findings: (1) they are not affected by anoxia; (2) changes in composition of the ionic environment do not influence the occurrence of either phase; (3) lowering the temperature does not abolish the positive phase but does reversibly abolish the negative phase (increase of the temperature of the retina to -85°C

and subsequent thawing changes the organization of the pigment molecules in the membrane and the fast signals do not appear); (4) fixation with formaldehyde or glutaraldehyde modifies only the shape of the fast phases of the ERP, but it abolishes the ion-dependent third phase; (5) after dehydration of the pigmented cells, only the fast-positive phase remains.

When the fast reaction of the retina to the pulse of light was compared with the response of a silicon cell connected with a circuit composed of resistance and capacitance representing the passive electrical properties of the biological material, the responses were indistinguishable (19, 48).

This apparently non-ionic nature of the first and second phase of the ERP led to consideration of the mechanisms of its generation in terms of solid-state electronics. In this perspective, the first act of generation of the visual sensation is the shift of electronic charges on the rhodopsin and possibly also neighboring molecules, followed by conformational changes that trigger the subsequent event of the visual excitation.

2. Chloroplasts

Photosynthesis can be viewed as coupled redox reactions; water is oxidized and carbon dioxide is reduced. The driving force for the reaction is, of course, solar radiation mediated by chlorophylls embedded in the thylakoid membrane of the chloroplast. Van Niel (49) first proposed this idea in terms of the oxidant, OH and reductant, H. In terms of solid-state physics, these entities are positive holes and negative electrons (50). If one assumes that chlorophylls are in an ultrathin liquid-crystalline-like lipid bilayer, absorption of light excites an electron to the conduction band and leaves a hole in the valence band. According to Katz (50), electrons and holes are free to move to effect reduction and oxidation, respectively. In other words, chlorophyll aggregate or chlorophyll dispersed in a lipid bilayer acts as a semiconductor (19). The photogenerated electron can be transferred to an electron acceptor. Similarly, the photogenerated hole can be combined with an electron donor. That chlorophylls and their host, the chloroplast, behave as a semiconductor has long been suggested by many authors, including Arnold and Sherwood (51, 52). Recently, owing to the interest in semiconductor electrochemical photocells for solar energy conversion (53), parallels between natural photosynthesis and semiconductor photoelectrolysis have been noted (1-5, 36, 53).

Considerations that energy conversion and transfer in chloroplast may involve conduction of electrons along common energy bands were first presented in 1938 (54). Consistent with the idea were the results on the temperature dependence of the conductivity of dried films of chloroplast (55), where conductivity changes were shown to obey Equation (2). In similar experiments, the photoconductivity of chloroplasts was found by Ichimura (56) to be also detectable in moist chloroplast films; however, the

current induced by illumination of these samples was smaller than in the dry ones. In contrast to inorganic semiconductors and pure chlorophyll layers, the rising phase of the current was relatively slow (minutes), and the conductivity response was biphasic.

Using a condenser method for photoconductivity detection, McCree (57) was not able to detect any signal from dried chloroplasts or from chlorophyll monolayers, dried proteins, green algae, and leaves. Because the method used made it possible to detect photoconductivity signals 10^3 times weaker than those generated by inorganic photoconductors, the author concluded that, if photoconductivity actually occurs in plant material, it could not be an efficient mechanism of photosynthesis.

Measurements of photocurrents generated in dried layers of chloroplasts of various plants (using a sensitive electrometer) have shown that the action spectrum of photoconductivity corresponds to an absorption spectrum with two exceptions (55): (1) in the region of the maximum absorption of chloroplasts, the action spectrum revealed a peak that was explained as due to increased surface recombination of carriers; (2) in some cases, the photocurrent was also generated when chloroplasts were illuminated with infrared light. The maxima of the infrared action spectrum were at 950, 1040, 1260 and 1550 nm. The presence of maxima in the long-wave region was interpreted as evidence of the existence of aggregation of chlorophyll molecules. It was also found that photoconductivity spectrum coincided with the absorption spectrum of the main pigments present in chloroplast membranes. Dark conductivity and conductivity in the illumination phase, in the temperature range of -20 to $+20^\circ\text{C}$, obeyed Equation (2) with conductivities and activation energy $10^{-13}\text{--}10^{-14} (\Omega\text{m})^{-1}$ and 1.72 eV , and $10^{-12}\text{--}10^{-13} (\Omega \text{ m})^{-1}$ and $0.05\text{--}0.2 \text{ eV}$ for dark and illumination conditions, respectively. The mobility of current carriers was estimated to be $10^{-5}\text{--}10^{-3} \text{ m}^2/\text{V} \cdot \text{sec}$.

The process of generation of free charges in chloroplasts and in the membranes of photosynthetic bacteria has also been investigated by means of measurement at microwave frequencies of the dielectric loss factor, which is equivalent to conductivity losses in the sample. In the study by Blumenfeld et al. (58), the dependence of the loss factor on wavelength of incident radiation of leaves and chloroplasts extracted from *Vicia faba* and *Sorghum sudanese* was investigated. The action spectrum of photoconductivity was similar to the absorption spectrum of chlorophylls a and b and probably of β -carotene. Although the action spectra of both leaves and chloroplasts were qualitatively similar, the action spectra of the leaves were higher by a factor of 5–7. When the chloroplasts were fresh and intact, a bipolar response was observed; when they were old or damaged (by heating, for example), the response was monophasic (58). A signal similar to that of chloroplasts was obtained in a model chloroplast system (59).

Bogomolni and Klein carried out combined measurements of photoconductivity at microwave frequencies, Faraday rotation, and electron-spin resonance in dried films composed of chromophores of photosynthetic bacteria (60). The photoconductivity signal

was generated by both negative and positive carriers; the former seemed to originate in thermal releasing of electrons from the primary acceptors of electrons, and the latter in hole movement in dimer chlorophyll cation radicals. The Hall mobilities of the charge carriers were of the order of $10^{-4} \text{ m}^2/\text{V} \cdot \text{sec}$. As in the previously described experiments, the action spectrum of photoconductivity was in agreement with the absorption spectrum. The data did not support a band-conduction model of conductivity in the photosynthetic structure. Instead, they seemed compatible with a model where photoliberated charges migrated by a hopping or tunneling mechanism (see Figure 4).

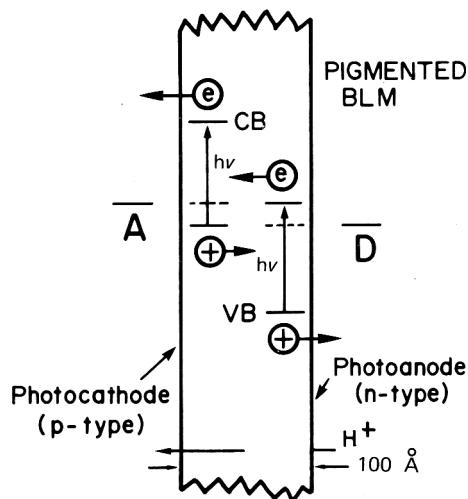


Figure 4. Electron tunneling in photoactive membranes. The operation of the double Schottky (or p-n junction) BLM cell is as follows: at each side of the BLM/solution interface, a space charge layer exists, which serves to separate photogenerated electron-hole (exciton) pairs. At the left-hand side, electrons move to the acceptor (A) at the interface where they effect a reduction, and the holes move to the interior of the lipid bilayer. At the right-hand side, separated holes move to the interface to cause an oxidation of the donor (D), and the electrons move toward the interior. Transmembrane electron movement is assumed to occur by tunneling (81-83). CB, conduction band; VB, valence band.

Microwave conductivity in reaction centers of photosynthetic bacteria has been shown to consist of two components having different rise-times (61). One, which may originate in the migration of electrons between quinones, rose rapidly (much less than 1 sec), and a second component which rose more slowly (about 20 sec). The amplitude of the fast component depended strongly on the hydration level, and it rose when the hydration increased. Other explanations for the origin of the fast microwave photoconductivity signal, considering its hydration dependence, is that water may influence the mobility of electrons in reaction centers in proteins, or that water may make the liberation of electrons to the conduction band easier. On the other hand, the slow component of the response may be interpreted as reflecting an accumulation of mobile

electrons in the electron transport chains, or changes of the charge distribution of the photosynthetic apparatus (61).

The emission of light, different from fluorescence and ordinary phosphorescence, from chlorella suspensions was discovered in 1951 (51, 52). One-tenth of a second after illumination, the intensity of the so-called delayed light was about 10^{-3} of the intensity of the fluorescent light from the plant, and about 10^{-6} of the intensity of the absorbed light. Even after several hours, it was possible to detect this extremely weak light by means of light-sensitive photographic material. That the shortest living components of the delayed light were of purely physical nature, and did not involve chemical reactions, was shown in a series of experiments. In experiments with chloroplasts where the intensity of the delayed light as well as its decay times were investigated down to -140°C , free radical reactions, and excitation and decay of triplet states were ruled out. This left, as the only plausible explanation, a mechanism involving formation of trapped electrons in a quasi-crystalline lattice of chloroplasts, followed by emission of light after detrapping and recombination with holes (62). The relatively fast rate of the decay of delayed light at -120°C was also interpreted as ruling out the involvement of enzymatic processes in generation of that light.

The depth of particular charge traps in the forbidden energy gap of a photosynthetic preparation may be revealed by investigating the dependence of the delayed light intensity on the temperature, or (at stable temperature) the time distribution of the emitted light (51, 52, 55). In a general semiconductor approach to the mechanism of the generation of the delayed light, the following sequence of events is suggested by the evidence. After absorption of light quanta of sufficient energy, the electrons are transferred from the valence band to the conduction band. As a result of imperfections in the chloroplast-chlorophyll crystalline lattice, empty electron traps exist in the forbidden energy band. A quantum of delayed light is emitted if, due to thermal vibration, a trapped electron is released to the conduction band and eventually to the valence band where it recombines with a hole.

This simple picture of the mechanism of delayed light generation has been modified by taking into account hole traps, discharging of the energy accumulated in traps by formation of radicals, and stable chemical species, and by placing the scheme in the electrochemical context of photosynthesis (51, 52). Although not discarded, this solid-state approach to the mechanism of delayed light generation is regarded by some as an oversimplification, and competitive approaches are reviewed by Malkin (63). Another approach to electronic conduction occurrence in chloroplast membranes is provided by experiments in which transient electric field gradients are brought about by fast movements of electrons across the membranes. These processes last less than 20 nsec and are accompanied by shifts in the absorption spectra of all photosynthetically active pigments (electrochromism) (64, 65). If this mechanism operates in normal physiological

conditions, one must take into account instantaneous flow of electrons from the inside of the thylakoid, where donors of electrons are located in its outer space. In addition, if Mitchell's mechanism of generation of ATP is correct, one would have to consider the flow of electrons along the thylakoid membrane transverse to the ion flow (66).

It has been shown that an outward current of electrons from chloroplasts was responsible for the transient induced electric field. In these experiments, chloroplast suspensions were illuminated with light flashes and small voltage changes were measured between two electrodes placed on the illuminated and dark sides of the chloroplasts. The risetime of the transient current induced in chloroplasts by the flash was less than 1 μ sec, indicative of its direct relation to primary events of photosynthesis. The electrode on the side of the light source was polarized negatively (67).

The results obtained in the above experiment are in qualitative agreement with observations of chloroplast electrophoretic mobility changes after illumination (68). When illuminated with continuous light, the chloroplasts became negatively charged and their mobility increased by an average of 15%. Chemicals that inhibited oxygen evolution abolished the light-induced electrical mobility rise, but uncouplers of photophosphorylation and cyclic electron carriers did not affect the increase of mobility during illumination. The data obtained may be interpreted as evidence for continuous electron flow across the membrane of chloroplasts during their photosynthetic activity. The importance of membrane integrity for the occurrence of the photo-induced increase of electrical mobility of the chloroplasts was also evidence for the possible involvement of solid-state charge transport across the membrane.

The very fast electric field induction after a strong light flash was observed in the leaves of the gout weed. The response was identical with the ERP found in visual pigments, as noted earlier (48).

The direct measurement of transmembrane potential difference generation by light in chloroplasts of *Pepperomia metallica* reported by Bulychev et al. (62) is consistent with the experimental findings described above. The rising phase of the field was smaller than 0.01 sec, and the potential generated was negative on the outside of the chloroplast. Although it is questionable whether a transthylakoid membrane potential was actually measured, the experiments demonstrate at least provisionally that a photovoltaic effect exists in the chloroplast, as has been observed in a different system by Luttage and Pallagy (39).

ELECTRONIC PHENOMENA IN BLM SYSTEMS

INTRODUCTION

The electronic properties of planar bilayer lipid membranes (BLM) may be studied either in the dark or the light. In the first case, the changes of conductivity of the membranes and the nature of the current carriers are of importance. These problems will be dealt with in the first part of this section. The phenomena evoked in BLMs by light include the generation of photopotentials, photocurrents, and changes of conductivity.

Owing to the original idea underlying the study of the properties of BLMs, the photoelectric phenomena in them are closely related to vision and photosynthesis. But a number of experiments have shown photoelectric responses in BLMs that were not dependent on specific pigments, but on the ionic composition of the bathing solution. There is also a group of experiments in which ultraviolet radiation was the factor eliciting the photoresponse.

Attempts were made to study the electrical responses resulting from illumination of model membranes consisting of lipids and pigments known to occur in biological membranes. Other studies involved membranes containing molecules mimicking biological molecules. This area of study has developed to the extent that spherical bilayers (liposomes) made of completely synthetic components now constitute an independent field. By achieving the photosynthesis-like energy conversion, it could be seen as a remote consequence of the studies that were begun on the reconstitution of chloroplast membranes in 1968 (69).

ELECTRONIC PROPERTIES OF BLMs IN THE DARK

1. The BLM System

The usual picture of a BLM interposed between two aqueous solutions consists of a liquid hydrocarbon phase sandwiched between two hydrophilic regions. The electrical properties of BLMs have been extensively investigated, which usually entails the measurements of membrane resistance (R_m , or conductance, $G_m = 1/R_m$), capacitance (C_m), potential (E_m), dielectric break-down voltage (V_b), and current/voltage (I/V) characteristics. Unmodified BLMs (i.e., BLMs formed from phospholipids or oxidized cholesterol dissolved in an n-alkane solvent in 0.1 M KCl solution) have typical intrinsic values of R_m greater than 10^8 ohms, $C_m = 5000$ pF, $E_m = 0$, $V_b < 200$ mV, and I/V curves obeying Ohm's Law. With a few exceptions, the interpretation of the results of these measurements has treated the BLM as electrically equivalent to an ionic resistor connected in parallel with a capacitor. The structure of the BLM is considered to be a thin slab of liquid crystals in two dimensions, having a fluid hydrocarbon core about 50 Å thick. The liquid-crystal portion of the BLM is an excellent insulator, but its electrical conductance can be drastically altered by incorporating a variety of compounds such as

iodine, valinomycin, 2,4-dinitrophenol, chlorophyll and its related compounds, and dyes (19, 70, 71). Of interest in this connection is a theoretical paper on solitary waves and solitons in BLMs (72).

2. Electronic Conduction Across BLMs

One of the most striking changes of BLM electrical properties was observed when I_2 and I^- were added to the bathing solution. The usually very high electrical resistivity of the BLM, which was in the range 10^{16} – $10^{17} \Omega\text{m}^2$, dropped by several orders of magnitude (19, 73-75). One possible explanation was that the membrane became more permeable to I^- or polyions of iodine (76). Another possibility was that the conductivity changes were due to electron conduction across the membrane (19). More specifically, conductivity changes due to charge-complex formation at the interface between the membrane and the bathing solution, and to the transfer of electrons across the membrane. After having measured capacitance and conductivity changes of BLM as a function of the frequency of the applied field and the concentrations of KI and I_2 in the bathing solution, Vodyanoy et al. (75) concluded that I_3^- was able to enter the BLM and act as an electron donor. It was also concluded that electrons in the membrane were transferred between donor and acceptor centers by hopping mechanisms. In similar experiments Boguslavskii et al. found that at the membrane-bathing solution interface, an exchange of electrons took place and that holes were the charge carriers inside the membrane (73).

To ascertain the role of ionic conduction in the observed drastic changes of conductivity of the BLMs under the influence of I_2 and I^- , tests using ^{131}I were carried out. Jain et al. (74) showed that conductivity changes of oxidized cholesterol-BLM were not accompanied by transfer of ^{131}I across the membrane. Moreover, the direction of the current of electrons was from the electron-donor side (I^- or thiosulphate) to the electron acceptor side (I_2). In a membrane of the same type of lipid, Karvaly et al. (77) also found that current transferred across the membrane was independent of ^{131}I (up to a specific flux level). From voltages generated by varying the iodine concentration in one of the compartments when $[I^-]$ was kept constant, and from I/V characteristics taken under the same conditions as with tracer measurements, Karvaly et al. concluded that, although some contribution of ionic conductivity could not be excluded, the current carriers across the membrane were electrons and that the membrane-bathing solution interface behaved as a semiconductive electrochemical electrode. The same conclusion was also drawn on the basis of voltages observed in systems where the concentration of the ferric ion in one of the compartments was also varied.

Feldberg et al. observed electronic conductivity across BLMs formed from glycerol mononucleate in n-hexadecane, and containing magnesium etiochlorin (78). The bathing solution contained a buffer and ferro/ferricyanide redox couples on both sides of the

membrane. The value of the redox potential of the couples could be changed by varying the ratio between ferrocyanide and ferricyanide present in the bathing solution. One of the methods of detecting the electron current flowing across the membrane was by measuring the open-circuit voltage when the redox potentials of the couples on both sides of the membrane were changed. Another method consisted of measuring the open-circuit voltage decay after a very short current pulse was injected from one of the electrodes. The third method was by measuring the changes in a steady-state voltage across the membrane when different steady currents were applied to the membrane. All three methods showed that the predominant charge carriers of the current across the membrane were electrons. The density of the current flowing across the membrane was shown to be proportional to the magnesium etiochlorin in the membrane. When the magnesium etiochlorin concentration was kept constant and the redox potential of the couples was changed, the resulting current across the membranes followed the magnitude and sign of these changes.

Another phenomenon involving the movement of electrons is known as electrostenolysis (19). The phenomenon can be described as follows. When a direct current is passed through a membrane (or barrier) of high electrical resistance separating two aqueous solutions, coupled electrochemical reactions occur on opposite sides of the membrane. Oxidation takes place on the side facing the negative electrode. Implicit in these reactions is a transverse movement of electrons across the membrane.

A dramatic demonstration of electrostenolysis in BLMs was seen in the following experiment. If an oxidized cholesterol BLM is interposed between a solution of cupric nitrate and sodium sulphide, a shining mirror is observed to cover the entire ELM area in 3–10 minutes. The brightness of the mirror has been found to depend on several variables, such as the concentration of $\text{Cu}(\text{NO}_3)_2$, the BLM resistance, the pH of the bathing solution, and the duration and magnitude of the applied voltage. For example, using the cell arrangement consisting of aqueous solutions of Na_2S and $\text{Cu}(\text{NO}_3)_2$ separated by an oxidized cholesterol BLM, mirror formation was observed within 100 seconds. If a pair of calomel electrodes were used to monitor the potential difference across the BLM, a voltage of 0–50 mV was detected when the mirror became visible. This voltage rose to about 200 mV when the entire BLM surface was covered by the mirror. The brightest mirror observed was at about 350 mV. When the BLM resistance was lowered by the addition of tetraphenylborate to the bathing solution, the mirror began to form within 30 seconds after adding $\text{Cu}(\text{NO}_3)_2$ to one side of the BLM. Concurrently, the membrane voltage rose quickly to about 150 mV and eventually leveled off at about 300–350 mV (23). Similar reactions were demonstrated in the BLM when the time dependence of BLM conductivity (current) in the presence of 0.1 M KCl and 0.1 M KI at 60 mV was measured. In the case of the KCl solution, the BLM current displayed practically no time dependency. Thus, the results obtained can be explained in terms of electrostenolysis in which the BLM served as a bipolar electrode, just as in the case of the

water/saturated KI solution interface. Upon passing a direct current, I^- was oxidized to iodine. The resulting product preferred the low dielectric BLM to the aqueous solution by a factor of 50. The enhanced conductivity of BLM under these circumstances has been attributed to the tendency of iodide to form polyiodides (76) which have a high solubility in the lipid phase, and which facilitate ion transport across the BLM.

3. Semiconductive Behavior of BLMs

As mentioned previously, an increase in conductivity with temperature may indicate semiconductivity as described by Equation (2). Membranes formed from oxidized cholesterol, both in the presence of substances forming charge-transfer complexes with membranes in bathing solution and without these substances, have been shown to obey Equation (2). Satisfactory agreement has been found in the temperature dependence of the conductivity between oxidized cholesterol BLMs and oxidized cholesterol in the solid state. A compilation of data for a variety of BLM systems using Equation (2) have been published (19).

PHOTOELECTRIC EFFECTS

Several comprehensive reviews on photoelectric effects in BLMs have been published (5, 19, 36, 79, 80). In this part of the review, some recently obtained data will be presented and emphasis will be put on the effects where electronic mechanisms seemed to be operating (6, 71).

1. BLMs Containing Visual Pigments

The first observation of the photoelectric effect in lecithin/cholesterol BLMs containing various carotenoids (all-trans-retinol, 9-cis-retinal, all-trans-retinol, β -carotene) was reported in 1969 (81-83). The photovoltages evoked in these membranes ranged from tenths of a mV to several mV, with rise times of about 1 second. Depending on the external conditions of the membrane (applied voltage, pH), the response was biphasic or monophasic. In later experiments, it was found that when $FeCl_3$ was used as an acceptor of electrons, and a membrane containing all-trans-retinal was illuminated with a short-duration light flash (0.8–3 μs), then a very fast electrical response of the membrane, similar to the R_1 phase of the ERP was observed. As in experiments on the dependence of the R_1 phase of the ERP of natural systems on pH, the R_1 observed in the BLM could be masked by increasing the pH. However, the enhancement of R_1 in natural systems with lower temperature could not be tested at subzero temperatures. Decreasing the temperature of the BLM system to 9°C seemed to increase the R_1 phase (81-83). The possibility that this response was due to proton transport processes was rejected for the following reasons: (1) the sign of R_1 was totally dependent on the location of the electron acceptors (the acceptor side became negative upon illumination); (2) the magnitude of the

voltage could be enhanced (up to 100 times) by addition of suitable electron acceptors; (3) in the presence of the high concentration of electron acceptors, the magnitude of R_1 was independent of the proton concentration gradient across the membrane; (4) at high buffer capacity, where the R_2 phase was completely abolished, R_1 could still be observed. Having excluded ionic mechanisms for the generation of the R_1 , Kobomoto and Tien put forth an overall picture of the sequence of events in the development of the ERP in both model and natural membranes (19, 79).

In an experiment where BLMs containing retinol were exposed on one side to a solution of $K_3Fe(CN)_6$, no biphasic response was found (84). Instead, a monophasic response was observed that depended on the relation of incident light, wavelength and absorption spectrum of retinol. A biphasic response was registered from BLMs containing vitamin A when the membrane was illuminated with a wavelength close to the absorption maximum of vitamin A. The photovoltage and photo current changes registered were on the order of a few seconds. Photo-oxidation of retinol and reduction of the Fe^{3+} present in solution was proposed as the explanation of the mechanism leading to photopotential development (84). This redox reaction has been shown capable of changing pH and of giving rise to the observed voltages. As the result of retinol oxidation, vitamin A acid is produced in the membrane and the response becomes biphasic.

The influence of light on the conductivity of BLMs formed of egg lecithin and cholesterol in heptane that contained rod outer segments, was studied by Fesenko and Lyubarskii (76). The time constant of the conductivity increase was about 30 msec after the light flash, and the rise was followed by a phase of increasing conductivity that was 16 times longer. Some times a photopotential of the order of 20 mV with a rise time of several msec was observed, but it was viewed as an artifact.

2. BLMs Containing Photosynthetic Pigments

This field of study is especially well developed. Motivation for this work comes both from attempts at understanding photosynthesis, and at constructing working models of efficient converters of light into electricity (2, 4, 80). In both cases, the efficiency of light transduction processes is the focal point of investigation.

The first observation of the photoelectric activity of BLMs containing chlorophyll and xanthophyll pigments (both extracted from spinach leaves or purchased from commercial sources) was reported in 1968 (69). The observed value of photovoltage was of the order of few mV, and its rising phase developed in less than 0.1 sec. The value of the first phase of the photocurrent density was of the order 10^{-15} A/m^2 . After the first phase reached its maximal value, it began to drop to a certain value and then kept increasing until the membrane broke.

The next step in approaching more closely the biological conditions was applying

asymmetric conditions across the membrane by the presence of oxidizing or reducing compounds, either on one or both sides of the membrane. To avoid the artifacts arising from a potential induced by pH gradients, buffers were used in the bathing solutions. The presence of redox gradients across the BLM dramatically changed their photoresponses. Especially high open-circuit photovoltages (of the order of 100 mV or more) were observed when Fe^{3+} ions were present on one side of the membrane, and 1,4-dihydroquinone or ascorbic acid was on the other side. The sign of the voltage generated at the Fe^{3+} (acceptor) side was always negative.

Quantum efficiency of chlorophyll-containing BLMs has been calculated to be very low (less than 0.005%). The action spectrum of BLMs was identical with the absorption spectrum of chlorophyll in the bulk solution, which indicated that the chlorophyll in the investigated BLMs did not form crystal complexes, which may be found when there is sufficient concentration of chlorophyll in a membrane-forming solution (19, 85).

In pursuing the direction of modeling the natural condition, the dependence of the photovoltage on the value of the redox gradient created across BLMs containing chloroplast extracts was studied, employing various concentrations of ceric/cerous ions, ferric/ferrous ions, and ascorbic/dehydroascorbic acid (86). It was found that the photovoltage generated across the BLM was proportional to the light intensity, and that at a given value of light intensity there was a limiting value of the photocurrent caused by increasing the redox potential gradient. The rise in temperature from 16°C to 25°C was followed by a drop in photovoltage, as shown earlier (19). However, the value of photoconductivity did not change with temperature, even when there were sufficient changes of the dark conductivity of the membrane. This experimental finding was interpreted as implying that the activation energy was zero or very small, and as a consequence, the photoconductive current could not be regarded as ionic in nature.

These observations, as well as more recent studies with cytochrome c-551 and flavine mononucleotide, were shown to be incompatible with an ionic mechanism of photoresponse generation. Instead, they appeared compatible with a model of electronic conductivity by the mechanism of quantum mechanical tunneling across the membrane (87).

In connection with electron tunneling, the following remarks are appropriate. Electron transfer and redox reactions in suitable membrane systems in which the energy storage is substantial, and for which the back reaction can be controlled, is a very important research area. One approach that has been pursued in our laboratory since 1968 is the pigmented bilayer lipid membrane system, in which the excited state transfers an electron to the acceptor through a tunneling mechanism. Figure 4 shows a simple diagram of a pigment on an ultrathin bilayer lipid membrane. The back reaction is prevented by the insulating lipid bi layer core. When the pigment is excited, the electron tunnels from

the acceptor side to the donor side, overcoming the energy barrier.

It has been shown that at the interface between the BLM and the bathing solution, photoredox reactions take place in which electrons may be transferred from excited chlorophyll to oxidants present in the solution (SmCl_3 or $\text{K}_3\text{Fe}(\text{CN})_6$ for example) or from reductants (ferrocytocrome c) to excited chlorophyll (88).

The ability of the BLM to respond photoelectrically has been shown to be dependent on a sufficient concentration of chlorophyll and carotenoids, and both pigments are regarded as essential in photocurrent generation. Chlorophyll is believed to act as a sensitizer, absorbing the light quanta and directing the electrons to carotenoids that span the membrane. Carotenoids provide a low electrical resistivity path for electrons moving across the membrane (89). However, this role of carotene pigment in BLM was questioned in experiments with liposomes, where electrons were shown to be transferred from chlorophyll across the lipid phase in the absence of carotenoids (90).

Experiments employing short impulses of light are especially useful in investigating the fast changes of the electrical event. Using different redox systems, pH gradients and applied external potentials, it has been found that the electrical response of the membrane after the light flash can be resolved into three components: the fastest one (risetime of 1–3 μsec), a slower component (about 20 msec), and the slowest part of the response (about 1 sec). Both the fastest and intermediary response were depicted as electronic in nature. The fastest response was interpreted as evidence of rapid charge separations; the intermediary response was explained as reflecting the process of exciton migration and transport of protons across the membrane. Electron mobility in the membrane was estimated on the basis of these experiments to be $10^{-5} \text{ m}^2/\text{V}\cdot\text{sec}$ (6, 19, 80).

Remarkably high photovoltages (155 mV) of the fast response (less than 10 μsec) were observed in a system consisting of a chlorophyll-ELM, and solutions of acetate chlorophyllin, and $(\text{NH}_4)\text{Ce}(\text{NO}_3)_6$. When FeCl_2 was added to the chlorophyllin-containing compartment, the photovoltage exceeded 200 mV (91).

Membrane-bound pigments of the photosynthetic bacterium, *Halobacterium halobium*, when incorporated in BLMs, make them photoelectrically responsive (92–96). Thus far, purple membranes of *H. halobium* has been investigated with the aim of testing the chemiosmotic theory of ATP generation, stressing the proton current across the membrane. However, electronic phenomena involving electron current transients across the membrane as a result of light absorption must be also operative in this system, as they are in the photosynthetic case (97).

3. Non-Pigmented BLMs

Photoelectric response of the lipid bilayer may be elicited in systems in which the bathing solution contains iodine and its derivatives. UV radiation from a nitrogen laser

(337 nm) was shown to cause a fast (much less than 40 sec) drop of the conductivity across the membrane (98). The photovoltage induced across the membrane was small, about 0.2 mV, and the current drop reached about 7×10^{-10} A. Generation of about 60 mV was observed from BLMs formed of oxidized cholesterol between solutions of ferric chloride and pure water, and illuminated with 365-nm light. When 100 mV was applied across the membrane, a rise in conductivity of 150–200 times was observed (99).

The current across the membrane diminished when the concentration of FeCl_3 was increased beyond 8×10^{-3} M. This was interpreted as evidence against the possibility that the observed changes of conductivity were due to a thermal effect. If this was responsible for the observed changes of conductivity, the conductivity should have increased with concentration because of enhanced absorption by the ions (99). Biphasic photovoltage changes were obtained across oxidized cholesterol BLMs dividing FeCl_3 and solutions when the system was illuminated with violet (365 nm) and ultraviolet (254 nm) continuous light. Immediately after the illumination began, a very fast negative potential was developed which was followed by a slow positive phase reaching a saturation level. When FeCl_3 was present on one side of the and iodide on the other side, a remarkably high (260 mV) photovoltage was generated. A significant difference was also found in the value of the negative current flowing across the membrane, depending on the polarity of the imposed potential difference. If the field was so directed that the negative charge carriers (probably electrons or hydrated ions) were expelled from the iodide-containing compartment to the one containing ferric chloride, the value of the current was significantly higher than if the field were oppositely directed (99).

4. BLMs Containing Various Molecules

Dyes embedded in BLMs present in the bathing solution have been shown to determine the occurrence and the value of the photoelectric response. A biphasic photovoltage was seen in a system composed of egg lecithin, and bathed with cyanine dye from one side (100). The action spectrum was similar to the absorption spectrum of the dye, and the photovoltage was proportional to the light intensity until a saturation value was reached. A similar dependence of the photovoltage upon the dye concentration was found, however after exceeding a certain concentration the photovoltage decreased. An extensive study of the photoelectric responses of similar systems containing various cyanine dyes was carried out (101, 102). Mechanisms involving intermolecular electron transfer in the photoresponse generation were assessed as unlikely for most of the cases. Instead, intermolecular charge shifts and translocations of dyes in membranes were suggested.

Using oxidized cholesterol-BLMs, and modifying the solution in one of the compartments either with methylene blue or rhodamine B, early investigators observed

photopotentials of up to 6 mV (6, 103, 104). The potentials were dependent on the spectral composition of the incident light and on the addition of KMnO₄ to the compartment containing the dye. It was suggested that a redox reaction took place at the interface between the membrane and the dye/KMnO₄ solution. Because the solution with modifiers was charged negatively, it was concluded that potassium permanganate acted as an electron acceptor, and the membrane itself as a hole conductor.

Hong and Mauzerell investigated a system composed of lecithin and cholesterol BLMs modified with magnesium octaethylporphyrin, using buffered solutions of potassium ferro- and ferricyanide (79, 98). They used a null current method which enabled them to differentiate between photovoltage dependent on redox gradients and photovoltage change arising from the movement of the porphyrin cation in the membrane. It was shown that continuous illumination gave rise to a voltage that depended on light intensity, applied voltage, and on the conductivity changes brought about by movement of the porphyrin cation. Using pulses of laser light and the voltage-clamp method in the same system, it was shown that the current rise time was shorter than 1 sec, and that photovoltage evolved exceeded 500 mV. A biphasic photocurrent was generated, and its first (extremely fast) phase depended on the capacitance established at the interface where a redox reaction between excited pigment and oxidant took place (80). At both interfaces, redox reactions occurred which were due to the charge carriers in the membrane and uncharged pigment molecules (79, 98).

Recently, Heubner (105) by means of an apparatus able to register potential changes in the nanosecond range, was able to show that a BLM separating a mixture of chlorophyllin with Na₂HPO₄, and solution of (Na₄)₂Ce(NO₃)₆, illuminated with a flash of light, generates a potential of the order of 30 mV in much less than 20 nsec. Estimated current densities in both the BLM and chloroplast membranes were about 10⁴ A/m² (64).

For efficient electron transfer and charge separation in green plant photosynthesis, a close proximity between a donor, such as chlorophyll, and an electron acceptor, such as quinone, is probably a prerequisite. To test this hypothesis, several investigators have synthesized covalently-linked porphyrin-quinone and porphyrin-carotene complexes as models for the initial photophysicochemical event in reaction centers of photosynthesis (106). Joshi et al. incorporated these newly available complexes into BLMs and found greatly enhanced photovoltage (302 mV) and photocurrent (22 nA), the highest reported values. Of all compounds studied, porphyrin-quinone (PQ) gave the most dramatic light response. The photoelectric action spectrum of the pigmented membrane closely followed the absorption spectrum of PQ, thereby providing strong evidence for the separation of electrons and holes in the lipid bilayer, and for the view that only the photons absorbed by the pigment were responsible for the observed light-induced redox reactions. To account for this finding, the pigmented BLM was considered to be an organic semiconductor separating two aqueous solutions. The membrane/solution contact

was analogized to that of a Schottky barrier except that the BLM system had two interfaces. One side of the membrane acts as a photocathode (p-type) and the other side as a photoanode (n-type). At the membrane-electrolyte interface, the aqueous solution played the role of the metal. When the quinone structure, an electron withdrawing group, was covalently attached to porphyrin (an electron donating group) as in PQ, the energy gap between the ground and excited states of PQ was narrowed, facilitating a push-pull effect of electron transfer. Absorption of light in the presence of appropriate redox agents led to a reduction on one side and oxidation on the other side of the membrane. When porphyrin was simply mixed with quinone of β -carotene in the membrane-forming solution, photoelectric effects were also observed, but the magnitude was lower by a factor of four as compared with the membrane containing covalently-linked porphyrin-quinone complex. Thus, in the PQ compounds, a more favorable orientation and closer proximity were attained between the donor-acceptor pair for light-induced charge separation, rather than being dissipated by other pathways such as fluorescence (6, 106).

In recent years, increasingly more attention has been paid to vesicular systems in the form of either liposomes or vesicles formed of surfactants (6). In one such system containing EDTA and methylviologen, a light-induced transmembrane reduction took place; EDTA in the inside of vesicles was oxidized and methyl-viologen-reduced (6). The reaction went against the redox gradient. In explaining this and other results, it was suggested that tunneling of electrons took place across the hydrocarbon-like core of the membrane (6, 107, 108).

In experiments involving egg lecithin membranes modified with various dyes and bathing solutions of reductants and oxidants, transmembrane photoreduction was observed (6, 71). However, the mechanism of the charge movement across the membrane was depicted as based on the process of diffusion of the dye anions across the membrane (109).

Redox reactions across the interface between the membrane phase and solution were also investigated in surfactant vesicles. Laser radiation was used as a light source. In an experimental system where the dye pyrene was incorporated in the hydrophobic phase of negatively charged dihexadecylphosphate vesicles, it was found that a hydrophobic environment lowered the ionization potential of the dye, and that electrons were expelled by the electric gradient arising from polar groups on the surface of the membrane (110). An electron transfer reaction from a donor (N-Methylphenothiazine or N-dodecylphenothiazine), present in the hydrophobic phase at the positively charged dioctadecyldimethylammonium chloride vesicle, to an electron acceptor, a surfactant derivative of tris (2,2'-bipyridine) ruthenium perchlorate anchored on the surface of that vesicle, was observed by Infelta et al. (111). A more extensive study on the vesicles formed of the same surfactant, and tris (2,2'-bipyridine) ruthenium cation and

methylviologen acting as donors and acceptors of electrons respectively, were carried out by Tunuli and Fendler (112). They investigated the efficiency of photoactivated electron transfer as a function of the location of the donor and acceptor on the membrane and found that efficient electron transfer occurred (with quantum efficiency 2.40×10^{-2}) if both donor and acceptor were attached to either the inner or the outer side of the vesicular membrane.

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

The existence of free electrons in both natural membranes and experimental bilayer lipid membranes (planar BLMs and liposomes) is a question still under discussion, in spite of considerable variety of data accumulated in the last 20 years. There are several reasons for these difficulties. Firstly, BLMs are extremely thin structures, and the physical theory of ultrathin layers is still in its beginning phase. Secondly, BLMs formed even in simple electrolyte solutions are relatively complex physicochemical systems, and an adequate description of all the significant interrelations presents many difficulties. Thirdly, because of the thinness of both natural membranes and BLMs, it is difficult to apply many of the physical techniques that have been successfully applied to thicker samples (see below). In spite of these difficulties, data have been accumulated to justify treatment of natural and bilayer lipid membranes as systems in which electronic phenomena play an important role.

ARE ELECTRONIC PROCESSES IN NATURAL MEMBRANES ADEQUATELY MODELLED IN BLMs?

1. Electronic Phenomena Without Light

The adequacy between some features of the behavior of natural membranes in dark, which may be interpreted as due to electronic processes, and supposedly electronic behavior of BLMs in the same conditions, is more questionable than that in the case of light-evoked electronic phenomena (113). Some of the phenomena that are the basis of their phenomenological electronic-like behavior, such as rectification and negative resistance, may be explained in terms of active transport of ions across natural membranes. On the other hand, some seemingly electronic phenomena may be alternatively interpreted in terms of changes in the conductivity of the membrane for charged aggregates, or conduction due to the presence of the products of hydrolysis of water in the BLM (19). However, there is little doubt that electron translocation processes occur during normal activity of biological membranes. Therefore, the more accurate question is whether these electron translocations play any significant role in the physiology of membranes. In this connection, it must be said that electron translocations across the membranes of mitochondria play a crucial role in establishing transmembrane pH gradients leading to ATP synthesis. In this way, electronic conduction in

mitochondrial and other proton-gradient-creating membranes appears to be connected with the basic process of bioenergetics.

With regard to information-transfer processes in biological membranes, it is possible that gating currents, discovered in nerve and muscle cell membranes, are electronic (114). Table 1 lists some experimental results on electronic behavior of natural and artificial BLMs obtained in the dark. To complete the overall picture of electronic effects, both in natural and artificial bilayer lipid membranes, estimations of the basic electronic parameters of mitochondrial, photosynthetic and artificial bilayer lipid membranes are compiled in Table 2.

2. Electronic Phenomena With Light

Table 3 presents a comparison of some of the features of the photovoltage generated in natural and artificial BLMs. On the basis of this comparison, it may be said that BLMs provide a realistic model of photoelectric phenomena in natural membranes. There are significant differences in the time course of the voltages. In particular, those measured in thylakoid membranes give much faster responses than those from BLMs. However, the main reason for this discrepancy lies in the limitation of the apparatus used in the experiments with the BLMs.

TABLE 1. Some Features of the Photovoltaic Response of Natural Membranes and Artificial BLMs

PHOTOVOLTAGE FEATURES	NATURAL MEMBRANES				ARTIFICIAL BLMs			
	Thylakoid	Ref.	Visual Receptor	Ref.	Containing Photosynthetic Pigments	Ref.	Containing Visual Pigments	Ref.
Amplitude (mV)	100	(116)	3	(41, 45-47)	<100	(6, 19, 36, 71)		(6, 71, 79, 81-83)
Latency (μs)	<40		Not detected (<10)	(45)	Not observed (?)	65, 72		(71, 79, 81-83)
Rise-time (ms)	<2.10 ⁻²	(116)	0.2	(45-47)	10 ⁻²	(36, 71)		(80, 98)
Temperature dependence	Decrease of photovoltage with increasing temperature		Decrease of photovoltage with increasing temperature	(45)	Photovoltage decreases with increasing temperature	(6, 71)	Photovoltage decreases with increasing temperature	(71, 87)
Adequacy between action and absorption spectra	Observed	(116),	Observed		Observed	(19, 71)	Observed	(71, 81-83)
Time-course of the sign of the response	In the fast light absorption induced phase the outside of the membrane changes negatively	(116)	Retinal side of the R ₁ of ERP changes after light flash	(41, 45-47)	Fast component negative on oxidant side	(36, 71)		(71, 79, 81-83)
Redox gradient dependence	Observed	(39, 117)			Observed	(19, 71)	Observed	(81-83)

TABLE 2. Some Representative Data on the Similarities of the Electronic Behavior of Natural Membranes and Artificial BLMs

EFFECT	NATURAL MEMBRANES	References	ARTIFICIAL BLMs	References
Increase of conductivity with rising temperature	Increase of 40–140% per deg $230 \pm 40 \mu\text{V deg}$ (a)	(28)	Increase of 40–118% per deg	(6, 19, 71)
Sensitivity to impurities	Electron donors and acceptors affect membrane conductivity	(1)	Observed increase of conductivity by a factor of about 103 when chloride ions in bathing solution are replaced by iodide ions (b)	(19)
Rectification	Observed rectification ratio 1:100 (c)	(1, 62)	Observed rectification ratio 1:22 (d)	(19, 36, 71)
Negative resistance	Observed	(11, 12)		
Thermoluminescence	Observed (e)	(62)		
Thermoelectric power	Observed $230 \pm 40 \text{ V/}^\circ\text{C}$ (a)	(22, 29-31)	Observed 80 V (f)	(19, 36)
Hall effect	Observed (g)	(1, 37, 60)		

(a) Cell membrane of the muscle of the frog, temp. range 0–35°C; (b) Phosphatidylcholine–BLM; (c) Squid axon membrane, ionic process; (d) Egg lecithin–BLM dividing solutions of ceric ammonium sulphate and ferrous chloride. Rectification ratio given for $V_p = 400 \text{ mV}$; (e) Spinach chloroplast and extracts from algae; (f) Chlorophyll–BLM, temp. range 15–40°C; (g) Net mitochondrial and in *Nitellopsis obtusa* cell membrane.

TABLE 3. Some Semiconductor Characteristics of Natural and Artificial BLMs

PARAMETER	NATURAL MEMBRANES				ARTIFICIAL BLMs	
	Chloroplast	Ref.	Mitochondrial	Ref.		Ref.
Type of dominant charge carriers	Holes	(60)	Electrons	(11, 12)	Electrons or holes	(5)
Density of dominant charge carriers (m^{-3})	10^{19} 8×10^{19}	(37, 60)	10^{23}	(116)	6×10^{23}	(6, 19, 71)
Conductivity mobility	10^{-5} – 10^{-3}	(11, 12)	5×10^{-6}	(73)	10^{-6}	
Hall mobility of ($\text{m}^2\text{V}^{-1}\text{s}^{-1}$) charge carriers	10^{-4}	(11, 12)	4.8×10^{-4}	(11, 12)		
Life-times of the charge carriers (5)	$\leq 5 \cdot 10^{-5}$	(11, 12)	$< 10^{-6}$	(73)		
Specific conductivity (Ωm^{-1})	10^{-15} – 10^{-14}		10^{-12}	(11, 12)	2.6×10^{-14}	(19)
Dominant mechanism of charge translocation	Tunneling and/or hopping	(60)	Band conduction in macromolecules and tunneling between macromolecules	(1)	Charge transfer at the interface and implied band conduction in the membrane	(19)
Energy gap (ev)	1.7–1.72	(11, 12)	2.6	(1)	1.1	(19)

This apparent similarity of the photovoltage from natural membranes and BLMs may be regarded as an evidence that the response is generated by the same type of electronic mechanism in both systems. Besides the features of the photovoltage response enumerated in Tables 1A and B, other observations made on natural membranes (or intact organelles) are worth recalling here. These include the occurrence of ERP signals in chloroplasts at very low temperatures, light evoked electron transmembrane transfer in erythrocytes, and photoinduced microwave conductivity increases in chloroplasts and in photosynthetic bacteria (42, 43).

One can distinguish two trends in the present stage of research on BLMs. The first is oriented at gaining more precise knowledge about natural membranes and mechanisms of their functioning, and the other is a technologically oriented approach. In this connection, it may be said that the photoresponses obtained in artificial BLMs surpass the biological membranes in many respects. Their quantum efficiencies (10^{-2}) are much closer to those of biological membranes (~ 0.5) than are the quantum efficiencies of BLMs containing chlorophyll ($< 5 \times 10^{-3}$). It seems quite probable that bio mimetic BLMs will evolve much faster in the direction of higher efficiencies than BLMs containing biological materials. Since a significant role is attributed to proteins in natural membranes, BLMs modified with a pigment and a protein may be regarded as a more advanced model of natural membranes. Consequently, it may be expected that further development of BLM models containing different kinds of photoactive compounds will provide more support for the existence of electron currents in BLMs and, indirectly, in natural membranes. However, a word of caution is in order. The BLM experiments have shown the necessary and sufficient conditions for an electrical response from BLMs that is similar to that observed in natural systems. It does not necessarily mean that the same process must exist in biological membranes. It is highly probable that similar relationships exist in BLMs and natural systems, but this can be proved only by building photoactive systems with a complexity more like that of natural membranes.

Both BLMs and natural membranes have been shown to respond like organic semiconductors to illumination, temperature changes, and doping with electron donors or acceptors. On the basis of these properties, models of organization and electronic function of artificial lipid bilayers and of the thylakoid membrane have been put forward. Despite these investigations and models, there is a need for more quantitative data concerning electronic effects in both types of membranes.

SOME NEW POSSIBLE EXPERIMENTS

1. A Suggested Hall Experiment with BLMs

The electrical and electronic properties of BLMs have been investigated thus far mainly in the transmembrane direction. The properties of the membranes in the plane of the membrane have been the subject of only a few studies. However, the properties in the

plane of the membrane in natural systems are also very important. Firstly, in electron transfer chains of photosynthesis and oxidative phosphorylation, electrons are believed to be arranged across, as well as in, the plane of the membrane. Secondly, in the direction across the membrane, the path of an electron is limited to the membrane thickness, whereas in the plane of the membrane, electrons may move much greater distances, provided that there are conduction paths present (intrinsic proteins), and driving potential differences available.

If a method of measuring and applying the potential difference in the plane of the membrane is found, a new class of electronic phenomena in the BLMs may be studied. The Hall effect and its variations such as photomagnetoelectric voltages might be investigated. The Hall voltage, V_H (perpendicular to the direction of the magnetic induction B), induced in a sample of the thickness of t_m (measured along the direction of B), is given by $V_H = R_H I B / t_m$, where R_H is the Hall coefficient of the material, and I is the current flowing through the sample perpendicular to B . If R_H of the BLM is taken to be $0.1 \text{ m}^3/\text{C}$, $B = 1 \text{ T}$, $I = 1 \text{ nA}$, the Hall voltage (measured in air) should be about 20 mV . V_H would be measured for polar groups of the BLM which, in air, should form the interior of the membrane.

Pursuing this line of consideration, it may be possible to investigate the magnetoelectric effects and the cyclotron resonance of charge carriers in membranes. The data obtained in these kinds of experiments would allow differentiation between electrons and ions as charge carriers (the difference in the frequency of the resonance), and the evaluation of effective mass of electrons and the geometry of conduction bands in the BLMs.

Another possibility for discriminating between electronic and ionic conductivity of BLMs, is the measurement of the transport of mass that accompanies the transport of charge. The planar geometry of BLMs would be an advantage in such studies.

2. Biomolecular Devices

The following experiments are formulated specifically to obtain an understanding of the principles, at the molecular level, governing molecular junction effects in electron-conducting ultrathin bilayer lipid membranes, and to apply these principles to the design and construction of devices having a lipid bilayer as their central components (115). The focus on the lipid bilayer arises from the fact that all biomembranes possess such a structure as their key element. As has been demonstrated to some extent, ultrathin lipid membranes less than 100 \AA thick, can function as gated channels, molecular diodes, photodetectors, and energy transducers. The choice of the compounds for incorporation into BLMs has been strongly motivated by the aim of modelling biomembranes. In the last few years, however, the interest generated by biomolecular electronic device s (BED) has provided new impetus. In particular, we believe that the bilayer lipid membrane

system can be used as a tool in BED research.

To construct a molecular diode, for example, using a TCNQ-TTF (tetracyano-p-quinodimethane-tetrathiavulvalene) complex, the classic monolayer technique of Langmuir and Blodgett could be attempted (19), with a monolayer of TCNQ as the acceptor on one side and a monolayer of TTF on the other side.

The idea of biofuel cells based on glucose oxidation has been known for some time but, to our knowledge, no one has developed a biofuel cell based on the scheme shown in Figure 5. The key element is, again, an ultrathin electron-conducting BLM, which serves as a bipolar electrode. For the arrangement proposed, an open circuit voltage in excess of 600 mV at a current density of 100 mA/cm^2 is predicted. Both voltage and current density will depend on the concentrations of species involved.

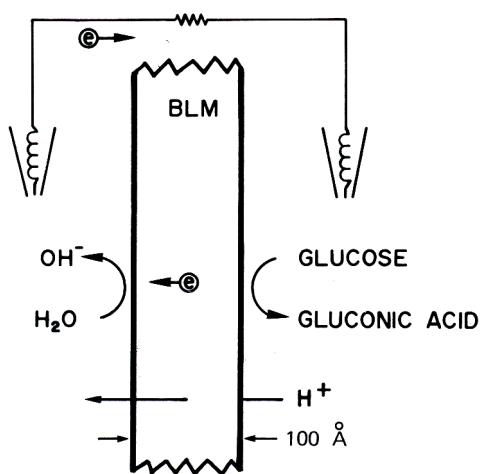


Figure 5. Biofuel cell using an electron-conducting BLM as bipolar electrodes (115).

3. Other Experiments

While the electrochemistry and photobiophysics of pigmented BLMs have been topics of study for many years, the interaction with microwaves has only recently been studied (65). Microwave energy has different effects than other forms of radiation, and it therefore can be expected to reveal different phenomena. One advantage is that the radiation damage to the system is extremely low. Thus, the dynamic properties of microwave interactions provide the possibility of probing states of motion in the

membrane and its adjacent interfaces. Finally, voltammetric techniques should be applied to both BLMs and natural membrane systems to probe the mechanisms of electron transfer and bioredox reactions (113).

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