Biological membranes

Living cells contain the ultimate in microelectronics: lipid bilayers carrying complex enzyme systems to perform sophisticated electronic and chemical functions.

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For solid-state physicists and engineers the "ultimate in miniaturization" would be to produce devices with structures that are about 8 or 10 nm across—about a tenth of the smallest scale that can currently be produced. (See PHYSICS TODAY, November 1979, page 25.) Biological systems, however, have, in a sense, solved the problems associated with such small microstructures. The fundamental unit of many cell functions, the lipid bilayer membrane (figure 1), is 4 nm thick; in regions where the membrane carries proteins it may be as much as 10 nm thick. Other elements of the cell, such as the microtubules that provide its structural framework, have similar dimensions.

These biological microstructures carry out a remarkable variety of functions, mechanical, chemical and electronic. In this article we will concentrate on the flow of electrons along and through the lipid bilayer membranes. Specifically we will consider some of the processes involved in respiration and photosynthesis.

Membranes

Much of the physiological and biochemical activity takes place not in the bulk cytoplasm of the cell but in membranes that are distributed throughout the cell and its organelles as well as forming their boundaries. These membranes consist of bilayers of fatty-acid esters (lipids) whose long hydrocarbon chains are repelled by the aqueous environment of the cell but are attracted to each other. The polar ends of the molecules (often consisting of phosphate esters) face the cytoplasm. In many cases protein molecules ("membrane proteins") are associated with these bilayers, often forming integral parts of the membrane. The electron micrographs of figure 2 clearly show many such membranes, some of which have a grainy appearance because they are associated with particularly large proteins. Figure 3 shows the structure of a typical membrane with embedded membrane proteins.

The membrane itself is impermeable to water and an excellent insulator. Many of the proteins in the membrane provide pores for active or passive transport of biologically important atoms and molecules through the membrane, thus maintaining or establishing electrical or chemical gradients. A nerve impulse, for example, consists of a propagating wave of electrical depolarization that derives its energy from a transmembrane ionic concentration gradient maintained by molecular ion pumps fueled by high-energy phosphate compounds such as adenosine triphosphate (ATP).

The membrane proteins have molecular weights between 100 and 250 kilodaltons, diameters between 4 and 10 nm, and are often composed of several subunits. They can be packed densely up to 10^{12} molecules per cm^2 or distributed sparsely, mixed together or organized in patches. Figure 4 shows examples of two such arrangements. The proteins are embedded in the membrane, and their amino acids that are in the hydrocarbon interior of the membrane are highly hydrophobic and make close contact with the lipid chains.

In most biological systems each distinct function is carried out by a separate molecule either alone or as part of a larger complex. In a few systems the same molecule can perform more than one function. For example, in the mitochondrion of the body cell one molecule serves both to transfer electrons to oxygen and to transfer protons across the membrane. (Strictly speaking, what is transferred is not protons but positive aqueous hydrogen ions; biochemists colloquially call these "protons.")

The electron-transfer systems we will discuss are the primary elements in a complex chain of energy-conversion mechanisms that, starting with a redox compound or a photon as a source, translate protons, generating an electrical potential gradient, then convert this gradient to a high-energy compound such as ATP, and finally utilize the ATP in many metabolic reactions and the maintenance of ionic concentration gradients.

Mitochondria

In eukaryotic cells (that is, cells with nuclei, as distinguished from bacteria and some other primitive organisms) respiration—the transfer of electrons from electron donors to reduce oxygen to water—takes place in mitochondria (on the left in figure 2). These are prominent organelles, about 2 microns in diameter, within the cells of every tissue and organ, separated from the rest of the cell by a membrane and containing many folded membranes as well. They appear to multiply independently of the remainder of the cell

Vesicles formed in water from lipid-bilayer membranes. The membranes were produced by hydration of lipids at low ionic strength. These vesicles are large enough for direct electrical measurements. Figure 1

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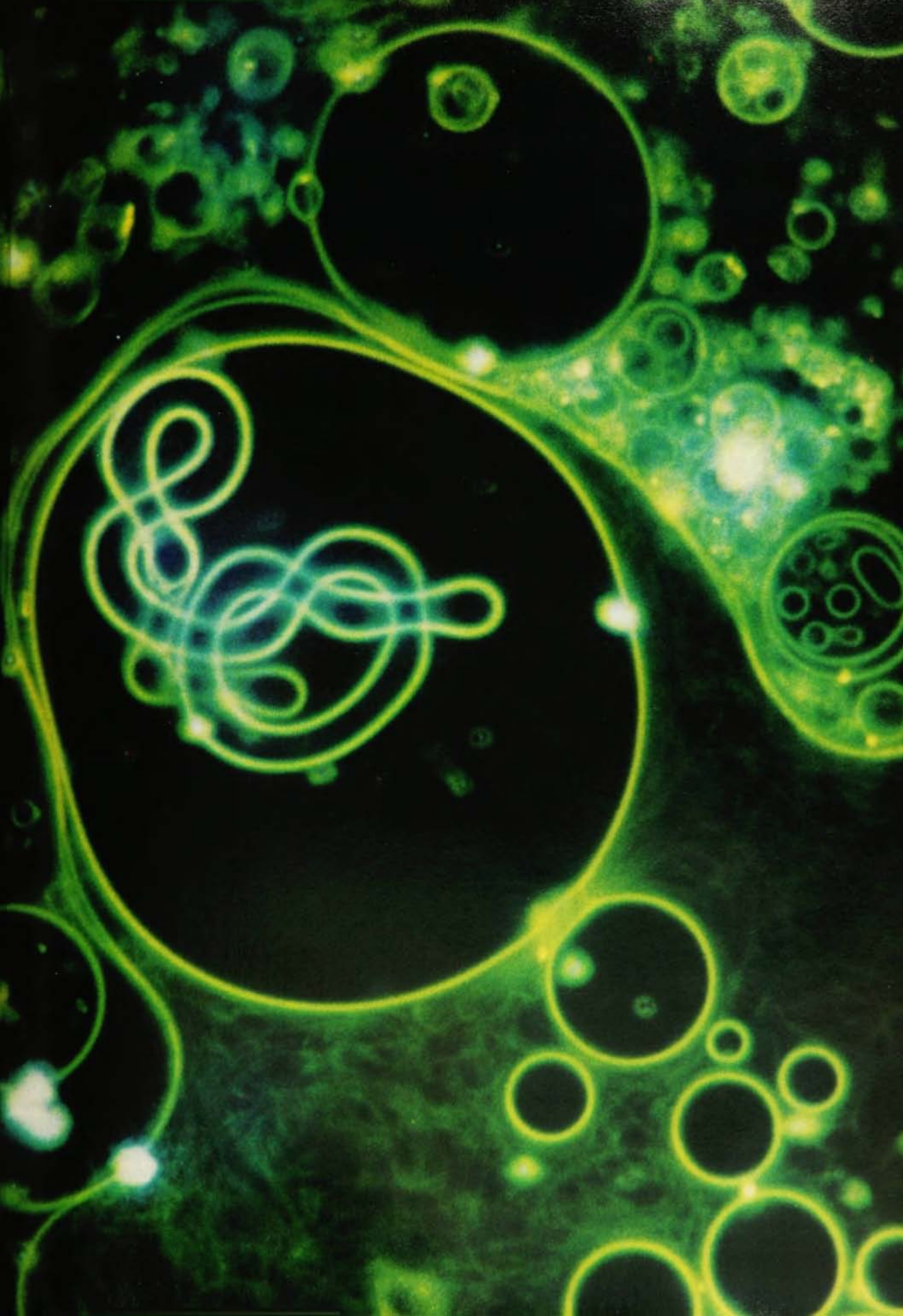
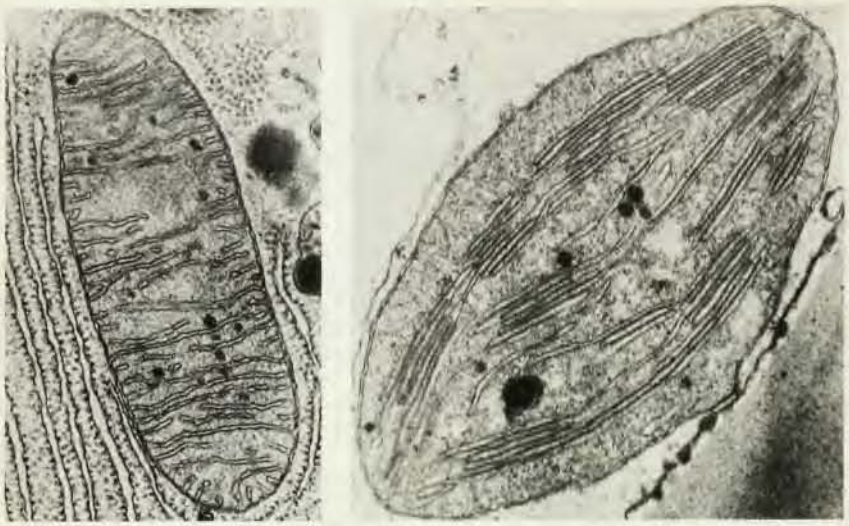




Cross sections of a mitochondrion and a chloroplast. These small, intracellular organelles are specialized to carry out oxidative phosphorylation or photosynthesis, respectively. Their membranes contain the molecular electron and ion-transfer components that are the subject of this article. The electron micrographs show areas of about 2 microns by 4 microns; they were taken by K. Porter and J. Antanavage. Figure 2

and carry some genetic information independent of the cell's (apparently with some differences in genetic code). It is widely believed that mitochondria are derived from once-free bacteria (similar in characteristics to *Paracoccus denitrificans*) that entered into an intimate symbiotic relationship with what then became the eukaryotic cell, increasing the efficiency of oxidative metabolism by over thirty fold.

The mitochondrion supplies "low cost" energy for the cell by burning foodstuffs in the presence of molecular oxygen transported to the cell by the blood, and utilizing the energy stored in the resulting transmembrane proton gradient for the formation of ATP. It does this in a series of steps involving an assembly of electron transfer components called "the respiratory chain" (figure 5). The electron carriers of the respiratory chain are arranged in three pools that consist of several membrane proteins whose redox potentials are roughly the same. Electrons are shuttled between pools by smaller, mobile components such as ubiquinone, which moves by diffusing through the membrane lipid, and cytochrome *c*, which diffuses along the surface of the membrane.

The components of each pool are near thermodynamic equilibrium.[1] The energy that is given up by the electrons as they drop from pool to pool is used to form molecules of ATP from ADP (adenosine diphosphate). Coupled to the electron flow is a proton flow, which—in at least one case—takes place through a transmembrane pump. Electrons and protons thus pass from the reduced form of nicotinamide adenosine dinucleotide (NADH) to oxygen, reducing oxygen to water and giving up energy to ATP in the process. A second electron and proton can enter the sequence midway, derived from the oxidation of succinic acid to fumaric acid.

The substrates of the respiratory chain, NADH and succinate, are derived from earlier steps in the metabolic process. Glucose, for example is converted to CO_2 and H_2O in several steps, each of which releases some of the energy stored in the molecule. First the glucose molecule is split into two molecules of lactic acid ("glycolysis"); next, molecules of CO_2 are sequentially split off from the lactic acid (the oxygen required is derived from water molecules) and the hydrogen is transferred to NAD and the respiratory chain (the "Krebs cycle"); finally the hydrogen is transferred to oxygen molecules to form water in the respiratory chain.

The overall chemical reaction of the respiratory chain is

$$4e^- + O_2 + 4H^+ \rightarrow 2H_2O$$

The electron flow along the chain (equivalently, the reaction rate) is quite rapid: Each carrier in a chain carries about 100–300 electrons per second.

Many of the molecules in the respiratory chain carry metal atoms, often found encased in porphyrin rings—as, iron is in hemoglobin. For example, the cytochromes (named for their intense pigmentation) contain porphyrin rings that carry iron atoms whose valence state can change from ferrous (Fe^{++}) to ferric (Fe^{+++}); iron atoms are also found in some proteins held by sulfur atoms in "iron–sulfur" centers. Other proteins contain copper, which transfers electrons by shifting between its cuprous (Cu^+) and cupric (Cu^{++}) states.

The protein components of these molecules, which are often quite large, appear to function to provide the metal atoms with the appropriate dielectric and chemical environment. The excess charge associated with the addition or removal of electrons from the metal atoms can, in some, of these cases, be stabilized by being spread around a large region of the molecule. As we shall see later, the proteins can also serve to adjust the energy levels of electron carriers so that the electrons can tunnel from one to the other.

Cytochrome oxidase

To illustrate some of the properties of the molecules in the respiratory chain, let us consider the cytochrome-oxidase complex, the last member in the chain, and the molecular complex that reacts directly with the molecular oxygen delivered to the cell by the circulating blood.

Cytochrome oxidase is a very large protein that spans the mitochondrial membrane. It extends about 5 nm beyond the membrane into the cytoplasm; it extends very little into the mitochondrion. (The membrane itself is about 4 nm thick.) The complex has seven subunits and a total mass of about 120 kilodaltons.

Two of the subunits (cytochromes *a* and a_3) contain iron atoms and two others (including one of the largest units) contain copper. The metal atoms are paired, as they often are in such complexes, but, interestingly, they are paired in heterogeneous iron–copper pairs instead of the more common homogeneous pairs.

The metal atoms are bound to their protein environment in a highly "covalent" way and with a high level of coordination so that electrons transferred to them can readily tunnel between atoms. Recent x-ray absorption studies have shown that the copper that is spin-paired to the iron of cytochrome a_3 has a local environment (that is, charge density) similar to that found in the "blue copper proteins" of some bacteria, while the other copper has a more covalent environment, possibly held by nitrogen and sulfur atoms of the protein. The iron–copper pair that involves cytochrome *a* appears to function as an acceptor for the electrons from cytochrome *c*.

The electrons removed from cytochrome *c* are then transferred to the other iron–copper pair, and by them to oxygen. The iron atom in cytochrome a_3 is the initial binding site for molecular oxygen. This atom appears to be so closely held to the copper atom that electron paramagnetic resonance as well as some portions of absorption bands are suppressed. Apparently the

local structure permits the iron and copper atoms (as Fe^{++} and Cu^{+}) to bind the oxygen molecule and transfer electrons to it; once the oxygen is reduced, the metal atoms bind the product in a peroxide bridge.[3] Protons from the surrounding medium serve to form hydrogen peroxide, still held by the iron–copper complex (now Fe^{+++} and Cu^{++}). Two more electrons from the rest of the oxidase complex then serve to form two OH^{-} ions, which are released into the surrounding water.

The iron–copper complex reacts avidly with oxygen. In fact, all these reaction are observed readily in the frozen state, for example at -125 °C, where the large molecules are essentially immobile and electrons must tunnel between them. At higher temperatures the reactions proceed rapidly, with oxygen passing through its four possible reduction states in microseconds, stopping momentarily at the two-electron reduced state to form the peroxide.

The structure of the surrounding proteins appears to be designed to store electrons and to deliver them to the bound oxygen molecule. Although the structure of cytochrome oxidase—as for other large molecular complexes—is not yet fully clarified, some of the important features appear to be shared with smaller, better-understood molecules such as cytochrome *c* and hemoglobin. One example of such a common feature is an iron (or sometimes other metal) atom bound into the porphyrin ring of a heme group. The ring contains four nitrogen atoms coupled with a system of conjugated double bonds that permits an extra charge to be delocalized over a fairly large area—effectively increasing the capacitance of the system—and helps to match the energy levels of the electron donors and acceptors.

Photosynthesis

In the biosphere the ultimate source of energy is, of course, sunlight, which is converted to chemical energy by photosynthesis. In fact, we now believe that all the oxygen in the atmosphere (on which all respiration depends) was produced by primitive photosynthetic organisms over several millions of years.

Plant cells contain special organelles, (at the right in figure 2), called chloroplasts, to perform photosynthesis. These, like mitochondria, are apparently derived from independent photosynthetic bacteria rather like the modern blue-green algae. In photosynthesis, electrons, instead of being released to a low-energy electron sink (molecular oxygen), are recycled. Their energy is raised in a special molecule, chlorophyll, by photons.

The photosynthetic apparatus in bacteria (such as the blue-green algae) appears to be more primitive than that found in eukaryotes. The central part is a "reaction center:" a large molecular complex (with a mass of about 90 kilodaltons) that spans the chromatophore membrane of the bacteria. The reaction centers are fairly densely packed in the membrane, as figure 3a shows.

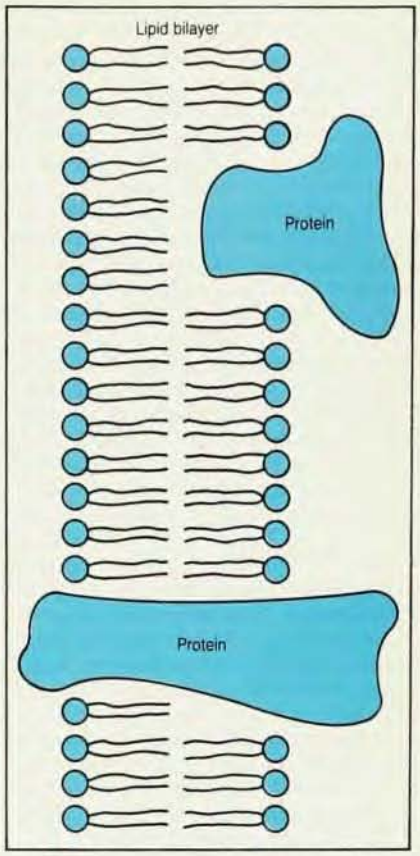
The reaction centers contain several subunits:[4]

- two bacterio-chlorophyll molecules that probably serve to collect the photons at the reaction center
- a bound pair of bacterio-chlorophyll molecules
- a pair of bacterio-pheophytin molecules (essentially chlorophylls without the magnesium atom at the center of the porphyrin ring)
- two quinone molecules and an iron atom.

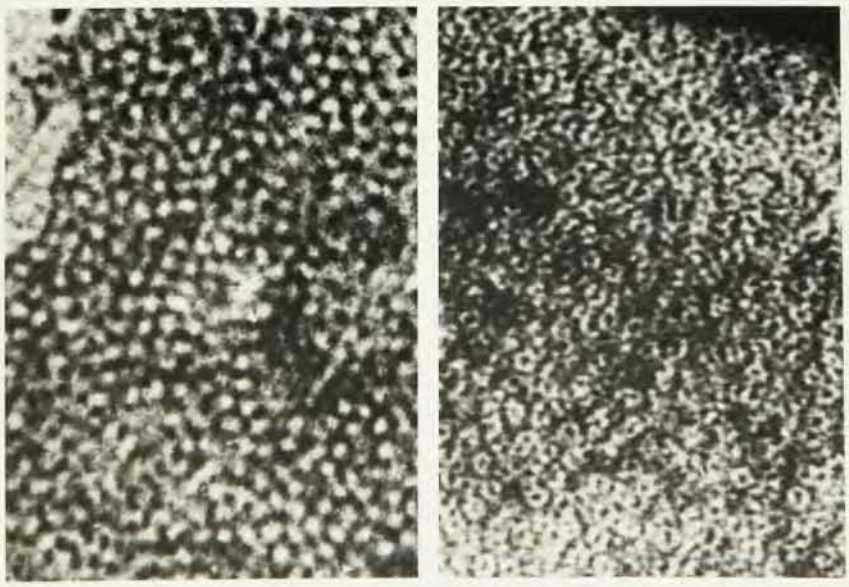
Their exact location and orientation in the reaction center are not known.

When a photon is absorbed by the reaction center its energy is transferred to the bound pair of bacteriochlorophyll molecules. The excited pair is then oxidized, losing an electron to the bacterio-pheophytin molecules; these, in turn, transfer the electron to the first of the quinone molecules, which passes it to the second. Apparently the chlorophyll dimer is near the center of the membrane while the quinones are near one of the surfaces.[5] The rapid transfer of charge to the quinones thus gives rise to a transmembrane potential.

From the quinones the electron is



Lipid bilayer containing membrane proteins. The proteins can serve as channels through the membrane for selected ions, as active trans-membrane pumps for specific molecules, as catalysts in chemical reactions, or as structural units in the membrane. Figure 3



Protein distribution in biomembranes. The electron micrograph at left shows photosynthetic reaction centers of the bacterium *Rhodopseudomonas spheroides* in a reconstituted membrane. The micrograph at right shows densely packed acetylocholine receptors in the membranes of the electric organ of the torpedo, an electric fish. These receptors are ionic channels that are opened by the binding of acetylcholine. They appear in this face view as rings composed of five or six subunits with a central opening, presumably the channel entrance. The electron micrographs were made by J. Antanavage. Figure 4

transferred to another complex—very much like that found in the respiratory chain—that uses the electron to reduce cytochrome *c*. The reduced cytochrome *c* in turn donates its electron to the reaction center where it returns to the bacterio-chlorophyll, thus completing the cycle. Because the binding site for the cytochrome *c* is, apparently, on the opposite side from the quinone molecules, the return of the electron stabilizes the charge separation. The quantum efficiency of the system is nearly 100%.

On its return path, the electron is associated with a proton, so that in addition to the cyclic electron flow there is a net transfer of protons from one side to the other. The proton gradient in turn drives other chemical reactions, such as the synthesis of ATP.

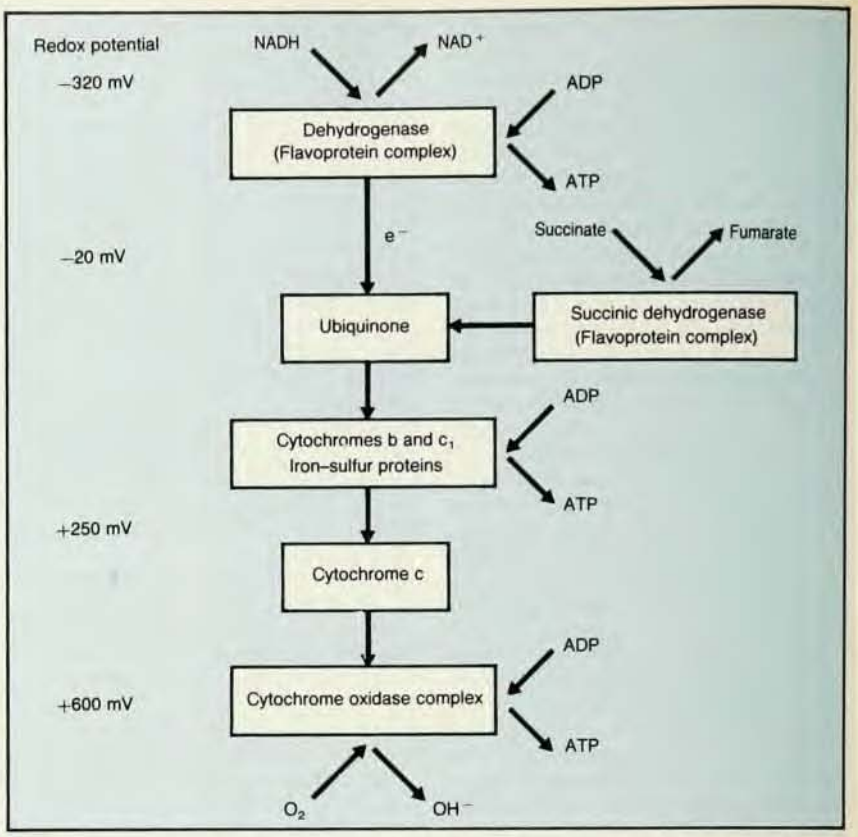
Electron tunnelling

As we have seen, many of the most fundamental metabolic processes involve the transfer of electrons between macromolecules—often between metal atoms held by the macromolecules. In ordinary chemical reactions, such a transfer would take place via a temporary chemical bond. In the case of the macromolecules, the distance between electron-carriers remains large enough (5–30 Å) that electrons must tunnel through the potential barrier separating the molecules.

This tunnelling process appears to be the central feature of the cellular microelectronics. Thermal agitation is constantly changing the parameters of these systems, and the electrons jump from one molecule to another when the conditions are right. Evolution appears to have designed the electron-carrying molecules and the membranes in which they are embedded so as to enhance the right conditions for jumping or to control the jumping.

The probability of tunnelling through a potential barrier is a function both of its height and width. The transfer of electrons is made easier when the molecules are close together, as they can be when one of the molecules is small (cytochrome *c* or the quinones, for example), or when the molecules have configurations that bring their carriers close together. The height of the barrier is determined by the electronic state of the molecules. The tunnelling is also affected by the relation of the energy levels on the two sides of the barrier: it is enhanced when the levels match up.

The potential energy of the electron and of the carriers is greatly influenced by the random fluctuations of the electrical fields produced by thermally agitated polar groups in the surrounding molecules. The reverse is also true: the electron jump exerts a sudden change of electrical forces on the surrounding molecules. Thus, the electronic motion is coupled to the nuclear (atomic) motion. The energy required to adjust the nuclear equilibrium positions from "electron on donor" to "electron on acceptor" (without, however, moving the electron) is called the "reorganizational energy." It is, for the sorts of systems we are considering, on the order of an electron volt.



Respiratory chain. The diagram shows the last stages in the conversion of sugars to CO_2 and water, as it occurs in mitochondria: the transfer of electrons (and hydrogen ions) to oxygen to form water. In the process several protons are transferred across the membrane; these can then return via the ATPase complex, generating molecules of ATP, which serves as an energy source for other biochemical processes. Figure 5

In figure 7 we show the potential energy of an electron for several configurations of the nuclei. For curve *A* the nuclear configuration is such that the electron is most stable at the donor, in configuration *B* it is most stable at the acceptor. The curves represent the equilibria prevailing before the electron transfer (*A*) and after (*B*). A net amount of energy ΔE is released in the transfer.

If the thermal energy is much smaller than the reorganizational energy, E_R, the nuclei rarely assume configuration *B* spontaneously. However, they can assume intermediate configurations, such as *C*, for which they only require an energy E^* on the order of the thermal energy. In this configuration the electron can tunnel through the potential barrier from the donor to the acceptor and the molecules can relax to configuration *B*, releasing[6] an energy $E^* + \Delta E$. The reaction rate in this case is given by the usual sort of expression:

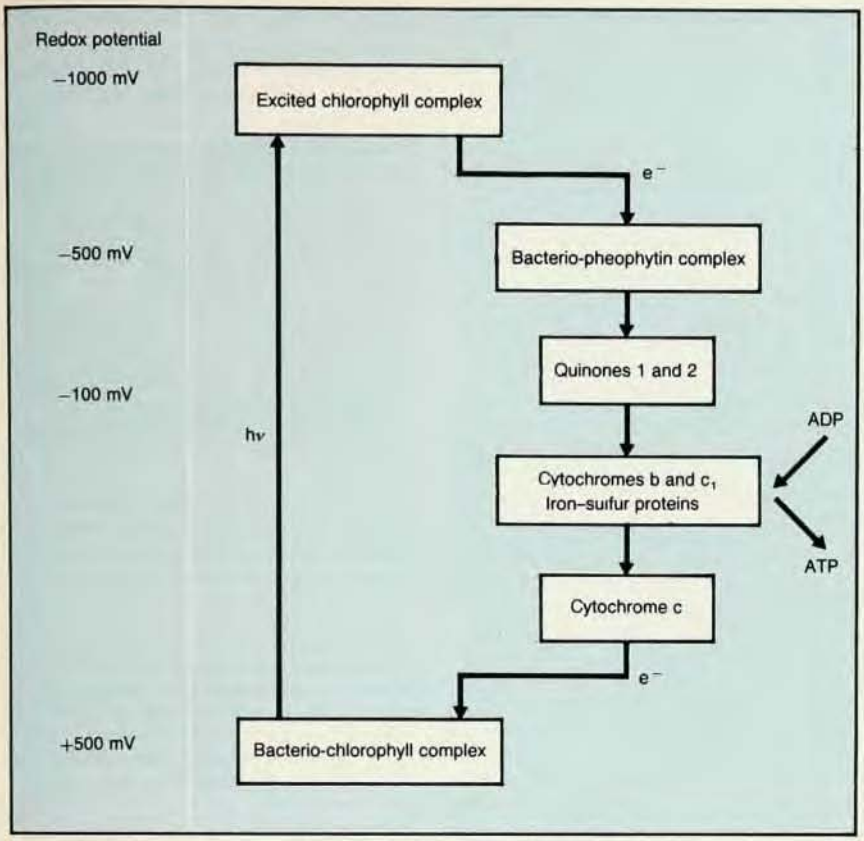
$$K(T) = Ke^{-E^*/kT}$$

where K depends on the probability that the electron will tunnel once the nuclear configuration is right. The exponential factor gives the probability that the nuclei will reach the proper configuration by thermal agitation. At low temperatures the tunnelling may still be possible, even in the absence of thermal agitation, if the wavefunctions of the nuclear states *A* and *B* both extend as far as configuration *C;* in that case nuclear tunnelling can occur without thermal activation, to be followed by electron tunnelling.

Experiments have shown that some rates of electron transfer in photosynthesis are temperature independent. In these cases, then, it appears[7] that low-frequency vibrational modes are not strongly coupled to the electron-transfer process.[8] If so, this is an achievement of "biological microelectronics" that has probably not yet been duplicated in ordinary chemical or artificial systems.

Control of electron transfer

Both photosynthesis and respiration are controlled by the metabolic needs of



Photosynthesis. The diagram shows the first stages in the conversion of sunlight to chemical energy, as it occurs in photosynthetic bacteria. The chlorophyll and pheophytin molecules, together with some others, make up the bacterial "reaction center," found in the plasma membrane of these bacteria. One molecule of ATP per photon is formed when protons transferred across the membrane return via the ATPase complex. Figure 6

the cell—whose survival, in fact, depends on that control. Respiratory control, for example, turns off electron transport when the energy needs of the cell are met, thus preventing waste of foodstuffs. Clearly, there is a feedback system that couples the energy demand of the cell back to the respiratory chain either in terms of chemical intermediates or in terms of trans-membrane potentials or ion gradients.

The rate at which the electrons are transferred by one of the enzyme systems of the sort we have described could be controlled by electric fields along the direction between the donor and acceptor. Such a field would, in effect, alter ΔE in figure 7. Because the activation energy is a function of ΔE, the field would affect the rate of electron transfer. In one model[6] of the transfer system, for example, in which the nuclei are assumed to behave as simple harmonic oscillators, the activation energy is

$$E^* = (E_R - \Delta E)^2/4E_R$$

where E_R is, again, the reorganizational energy. Where ΔE has the same value as E_R the activation energy vanishes (and, incidentally, the reaction rate becomes temperature-independent). The rate at which E^* varies with ΔE also vanishes, and small variations in ΔE can thus not be used to control the kinetics of the reaction. For such systems other means of control must be found. On the other hand, if ΔE is small compared to E_R (the situation shown in figure 7), E^* varies linearly with ΔE and the reaction rate also depends on ΔE.

In more complex systems, of course, the control can take on a greater variety of forms. Just what the control mechanisms are in biological systems is difficult to determine: so many parameters affect their behavior that it is difficult to untangle their effects. What is needed are more precise probes of living systems and some simpler systems in which to study the phenomena.

Membrane reconstruction

Most natural membranes contain proteins acting in parallel and having many different functions. Moreover, mitochondria, chloroplasts, and bacteria are too small for reliable direct electrical measurements. For these and other reasons, the development of methods for the isolation of individual membrane proteins and their insertion into lipid bilayers has led to new insights into specific mechanisms and structures. Reconstituted membranes are in the form of either small (300–1000 Å diameter) vesicles or planar bilayers that separate two aqueous phases large enough to allow direct electrical measurements. Experimenters have been able to insert a great variety of membrane proteins, including members of the mitochondrial electron-transport chain, into the membrane of small vesicles. These protein-carrying vesicles exhibit activities such as oxidation-coupled protein transfer and oxidative phosphorylation. Through such studies the central ideas of energy coupling and transformation in the "chemi-osmotic theory"[9] can be tested experimentally. According to this model the major function of systems such as the reaction centers or the respiratory chain is, as we have suggested, to produce a proton gradient across the membrane. This proton gradient then drives other enzyme systems, in particular the ATP-generating complex: every pair of protons that flows through the ATPase complex produces one molecule of ATP. In photosynthetic organisms the proton gradient can also be used to reduce NAD^+; the resulting NADH serves as a general reducing agent (for example, driving the Krebs cycle in reverse).

Lipids can be made to form monolayers on the surface of water: One places a drop of lipid dissolved in an organic solvent such as hexane on the surface of water; the drop spreads to form a film and after the solvent evaporates one has a layer of lipid on the water (hydrocarbons pointing up). One can coat such monolayers onto each side of a plate with a hole in it, forming a bilayer membrane across the hole.) Such bilayers are by now fairly well studied objects. They have a capacity of 0.5–0.7 microfarad/cm^2, a typical resistivity of 10^8ohm cm^2 and an elastic constant of 90–100 dynes/cm. The lipids move about freely in the plane of the membrane and occasionally flip around to the opposite side.

Lipids dispersed in water naturally form globules of lipids with their polar ends pointing out or vesicles enclosed by bilayer membranes. The size of the vesicles can be controlled by adjusting the ionic strength of the water. Figure 1 shows a collection of large vesicles formed in this way. Such a vesicle can be turned into planar membranes by attaching it to an opening in a partition and then breaking the vesicle open on one side with an electric shock.

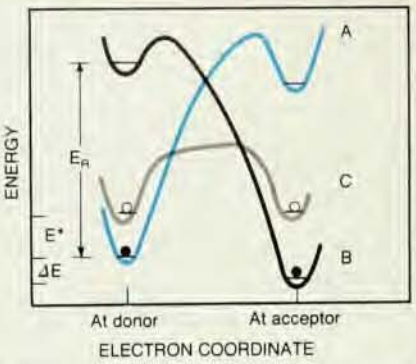
Several groups have been able to produce protein-carrying membranes and to study their electrochemical properties. Among the enzyme systems studied in this way are cytochrome oxidase, bacterial reaction centers[10] (see PHYSICS TODAY, September, page 19), rhodopsin, acetylcholine receptor[11] and several other ionic chan-

nels derived from bacterial and mitochondrial membranes. Some of these channels have voltage-dependent conductances and display all the electrokinetics of electrically excitable membranes, including action potentials.[12] In many cases one can observe the opening and closing of individual channels; the statistics of this channel activity give clues about the gating mechanisms for the channels. In planar membranes containing cytochrome oxidase one can directly measure the currents and potentials associated with the transfer of electrons from cytochrome *c* to molecular oxygen.

Membranes containing reaction centers are, of course, particularly interesting for studies of photosynthesis. The photo-induced currents and potentials observed in bilayers containing reaction centers agree well with the electron transfer mechanism derived from spectroscopic data. Such membranes give currents in response to steady-state illumination that consist of an initial peak, a smaller steady-state current, and a reverse current on cessation of illumination. The action spectrum matches the absorption spectrum of isolated reaction centers.

With excess reduced cytochrome *c* added to one side of the membrane as an electron source and additional ubiquinones as acceptors in the bilayer, the integrated peak current represents the transfer of several electrons per reaction center from the cytochrome *c* to the ubiquinones. The integrated peak current is determined by the number of added quinones. The steady-state current results from the oxidation of the quinones by O_2, which maintains a steady supply of electron acceptors. The reverse current is due probably to transmembrane equilibration of the anionic semiquinone.

The total charge transferred, as measured from the current integral in response to a single turn-over laser flash, matches the number of reaction centers in the membrane as estimated from the reaction center concentration of the membrane-forming solution. The membranes contain approximately 2.5×10^9 reaction centers/cm^2. The amplitude of the flash-induced potential change is commensurate with the membrane capacity and the integral of the current due to a single flash. Each center can transfer about 5×10^9 electrons per second, and with about 2×10^{11} centers per cm^2 the membrane can reach current densities of about 150 A/cm^2 in response to brief intense flashes of light, but under physiological conditions the electron-transfer rates are much lower. However, a densely packed (6×10^{12} centers per cm^2) oriented monolayer of reaction centers could theoretically give transient currents of



Electron transfer. The graph shows the potential energy of an electron as a function of position for three configurations of a pair of molecules—an electron donor and an electron acceptor. In configuration *A* the most stable state has the electron bound to the donor. In *B* the electron is most stable at the acceptor; this state has the lowest energy. Configuration *C* permits electron tunneling between the two sites. Figure 7

up to 600 A/cm^2. If electrons could enter and exit the reaction center from metal or semiconductor layers with appropriate energy levels in contact with the monolayer, one might be able to obtain large steady-state currents as well. Many such layers coupled in series would generate large potentials and power.

Preliminary experiments with condensed monolayers of reaction centers sandwiched between two transparent evaporated metal layers have demonstrated the feasibility of this approach, and although no practical applications are foreseen, these experiments may be of use in the design and construction of energy-conversion devices.

Such studies illuminate the function of the various components of biological microstructures. The mechanisms for producing the functions, are, however, far from being fully understood. To a great extent, this is so because the structures of the molecular machines have not yet been resolved at the atomic level. In most cases, we have only a vague outline of the size and arrangement of the subunits at resolutions not much below 2 nm.

Membrane proteins do not readily produce crystals of high quality suitable for x-ray diffraction, precluding the use of that powerful tool for structural analysis. In some cases, however, experimenters have been able to produce fairly high-resolution maps. For bacterial rhodopsin (which acts as a light-driven proton pump) both the amino-acid sequence and the folding pattern through the membrane are known. At present, we have a fairly complete understanding of the correlation between structure and function down to the atomic level for only one protein, gramicidin, a simple cation-selective channel.[13] But although detailed structural data are an indispensible prerequisite, they do not guarantee full mechanistic understanding. For example, we do not fully understand how hemoglobin works although we have a very clear picture of its structure.

The biological microstructures we have discussed perform a variety of chemical and electronic functions. Other biological systems, whose structure and function are as yet only vaguely understood, serve as gated channels, power supplies, pumps, receptors, effectors and transducers. In an individual cell they perform all the functions necessary to sustain life. Assembled into a brain, for example, they perform as incredibly powerful information processors. Physicists and engineers concerned with building ever smaller devices can look to them for inspiration.

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References

1. F. Wilson *et al.*, Ann. Rev. Biophys. Bioeng. **3**, 203 (1974).
2. T. Frey *et al.*, J. Biol. Chem. **253**, 4389 (1978).
3. B. Chance *et al.* in *Cytochrome Oxidase, Developments in Biochemistry* **5**, T. King, K. Okunuki, Y. Orii, B. Chance, eds., Elsevier, New York (1979), page 353.
4. W. Sistrom, R. Clayton, eds., *The Photosynthetic Bacteria*, Plenum, New York (1978).
5. J. Katz in *Light-Induced Charge Separation in Biology and Chemistry*, H. Gerischer, J. Katz, eds., Dahlem Konferenzen, Berlin (1979) page 331; J. Jackson, P. Dutton, Biochim. Biophys. Acta **325**, 102 (1973).
6. R. Marcus, J. Chem Phys. **43**, 679 (1965).
7. J. Hopfield, Proc. Nat. Acad. Sci. USA **71**, 3640 (1974); J. Jortner, J. Chem Phys. **64**, 4860 (1976).
8. A. Kuznetzoff, N. Sondergård, J. Ulstrup, Chem. Phys. **29**, 383 (1978); A. Sarai, Biochim. Biophys. Acta **589**, 71 (1980).
9. P. Mitchell, Biol. Rev. **41**, 445 (1966).
10. N. K. Packham, C. Packham, P. Mueller, D. M. Tiede, P. L. Dutton, FEBS lett. **110**, 101 (1980); M. Schonfeld, M. Montal, G. Feher, Proc. Nat. Acad. Sci. USA **76**, 6351 (1979).
11. H. Schindler, U. Quast, Proc. Nat. Acad. Sci. USA **77**, 305 (1980).
12. P. Mueller, *The Neurosciences, Fourth Study Program*, F. Schmitt, F. Worden, eds., MIT Press, Cambridge (1979), page 641.
13. D. Urry, Proc. Nat. Acad. Sci. USA **68**, 1907 (1971). □