PHYSICAL BACKGROUND OF EXCITABILITY: SYNTHETIC MEMBRANES AND EXCITABLE CELLS

Aleksander T. Przybylski

NFCR Laboratory at the Institute for Molecular and Cellular Evolution, University of Miami
Coral Gables, Florida 33134

INTRODUCTION

The value of biomimetric models is unquestionable. Beyond their heuristic role, they have also a practical aspect. The studies of reassembled biological material models preceded those of fully artificial materials. We shall in the first three sections review very briefly the irritability (excitability), which is common not only in the animal realm but also in living matter in general, and then discuss the details of our new findings on excitability of synthetic artifical cells.

The electrophysiological properties manifested by plant protoplasmic droplets were very helpful in finding a way to answer whether artificially assembled cells possess excitability. The answer was positive (Takenaka et al., 1971). About one hour after their formation, reassembled protoplasmic droplets displayed membrane and action potentials.

In 1973 the first findings on excitability of synthesized proteinoid cells were reported (Ishima and Fox, 1973). Three findings were further expanded (Ishima et al., 1981; Przybylski et al., 1982). selective permeability and osmotic properties (Fox et al., 1969; Fox and Nakashima, 1980) and bilayer membranes made of proteinoids (Fox et al., 1978) give us an approach which allows an understanding of the origin of excitability. The present paper aims to point out some data obtained on artificially produced proteinoid cells with an attempt to find the very basic physical laws underlying the origin of excitability.

EXCITABLE MEMBRANES

The membranes fulfill two main functions: (a) creation of the barrier between the cell's internal contents and the external environment, and (b) generation and propagation of excitation. Both of these functions are intrinsic in understanding the origin and function of the first living entity. Excitable membranes, beyond natural ones, comprise two types of artificially made micelles: laminar (bilayer molecular membranes) and spheroidal (vesicles or microspheres). The underlaying mechanism of the origin of excitable membranes made of such compounds as phosphatidylcholine (lecithin), cholesterol, retinal, etc., under conditions of mechanical agitation (Goldacre, 1958; Rutten, 1971; Tien, 1974) is their energetically favorable interface orientation.

A decrease in bifacial energy takes place during bilayer membrane formation, and, therefore, the micelle is more stable than a separate molecule. The corresponding entropy change ΔS is given by:

$$\Delta S = d(\Delta F_i)/dT_i$$

where

F_i : standard free energy,

T : absolute temperature (Tien, 1974).

A membrane potential in a bilayer membrane-liquid system is due to at least one of the following potentials:

distribution potential
Donnan potential
redox (electrostenolytic) potential
electrokinetic potential
photoelectric potential

and depends on the chemical composition of the membrane and the liquid phase of the system. The contribution of each of the above potentials is different. Physical factors resulting in membrane conductance comprise a series of events: interphase surface charge, ion activity, transmembrane ion gradient, oriented dipoles at membrane surface, dielectric constants, and intramembrane ion mobility. In turn, these conductance properties of the membrane with the associated ionic gradients determine the steady-state potential as well as dynamic potential changes, either induced (e.g., by current stimulation) or spontaneous. The spontaneous potential oscillations are observed under strict conditions of membrane composition, preparation, salt gradient of the liquid phase of the system, pH, and temperature. If photosensitive dyes are present in the membrane, the electrical behavior of the membrane

is determined also by factors responsible for trapping of photons, formation of carriers and charge separation.

MECHANISM OF POLARIZATION CHANGE OF THE MEMBRANE

The membrane resistance is the first contributing factor involved in the observed membrane polarization changes.

Resistance of the Membrane

The resistance of the membrane is its crucial feature, both in regard to barrier and permeability function as well as to charge separation. The above mentioned factors contributing to membrane polarization are evidently involved in determination of the membrane resistance. The membrane resistance is

$$R_m = E_m R_i / E_i - E_m,$$

where

R_i is the series resistance, E_m --the voltage across the membrane, and E_i--the calibrated input voltage. The membrane capacitance (of oxidized cholesterol BLM, e.g.) is related to voltage (White, 1970)

$$C_m = C_o + bV_a^2 ,$$

where

C_o : the capacitance at zero voltage,
b : temperature constant,
V_a : applied voltage.

The change of BLM capacitance is dependent on electrolyte concentration. Its minimum has been detected for uni-valent ions in electrolytes such as KCl, NaCl, at a concentration of 0.1 M, and in the case of di-valent electrolytes such as $MgSO_4$ it has been found at 0.025 M concentration (Rosen and Sutton, 1968).

The bilayer capacitance C_m can be expressed also as a dependence:

$$C_m = t/R_p \ln (E_o/E_t),$$

where

t : time (sec),
E_o, E_t : voltage at $t = 0$, and t, respectively,
R_p : leakage resistance, equals $(R_m R_i / R_m + R_i)$.

When $E_m/E_o = 1/q = 0.37$ (q : charge) the time constant is $\tau = R_p C_m$.

This constant is a useful measure of the temporal membrane characteristics.

The resistance of the membrane is linear when the concentration of electrolytes across the membrane is the same. If different, both types of membranes (laminar and spheroidal) display a nonlinear characteristic with a negative resistance.

Because of the high dielectric constant of the bilayer membrane and very small interlayer distance, it can hold a high charge (of an order of 10^5V/cm). The lipid-like substances are characterized by high resistance, and protein presence of lipid-like membranes diminishes resistance considerably. It has been found that an addition of natural protein (excitation-inducing material) reduces membrane resistance to as low as 10^1 ohm/cm^2 from an initial value of 10^6 - 10^{10} ohm/cm^2 (Mueller and Rudin, 1968).

The Electronic Process in Membranes

The possibility that mechanism analogical to electron conduction in proteins might play a role in biological processes was considered by Szent-Györgyi over 40 years ago (1941).

Studies of such processes as vision, photosynthesis, chemical respiration, carcinogenesis and nerve excitations on submolecular and quantum levels are being better understood in terms of the underlying mechanism of these processes.

We can consider BLM as an organic semiconductor; however, the current carriers involved in the dark conductivity of unmodified BLM are most likely ions rather than electrons and holes. But the conductivity of BLM can be raised several orders of magnitude.

If sufficient energy is absorbed by the membrane, electrons and holes can be generated. To emphasize similarities between the living system and the semiconductor device, an analogy between biological semiconductors and inorganic semiconductors is given in Table 1.

There are more considerations and experimental data on electronic processes in the living system. Several possible mechanisms such as electron tunneling and hopping are discussed.

The high probability and significance of the tunneling mechanism in biological processes is within the range of 10^9 to 10^{-9} s^{-1} with slight changes only in interstate distance or energy between states (Lewis, 1982). This means that the occurrence of an event or its absence is conditioned by slight changes of energy or geometry of the molecule.

Table 1. An Analogy Between Biological and Inorganic Semiconductors

Component	Inorganic semiconductor	Biological semiconductor
Base material	Covalent-bonded Crystalline phase (e.g., germanium crystal)	Hydrophobic hydrocarbon phase (e.g., lipid bilayer)
Electron donor	Group V elements (e.g., As, Sb)	Bio-reductants (e.g., cytochromes, ferrous ions, and H_2O)
Electron acceptor	Group III elements (e.g., Ga, In)	Bio-oxidants, (e.g., ferredoxins, quinones, and H_2O)
Electron pathway	Crystal proper	Conjugated hydrocarbon chain and ring systems
Connector	Metallic wire	Electrolyte solution

(Tien, 1974)

The role of the membrane is significant because of its ability to orient active molecular groups and direct charge transfer and electron transport.

These processes are determinated by a descending sequence of energy levels and the existence of an electric field. This includes the energy of water solvation. Eley et al. (1979) established that in a dry state conduction is determined by holes and by electrons as water absorption occurs. The water-protein interaction involving charge carriers determines the electrical properties of the membrane. In the case of the artificially assembled protocell of thermal polymers of amino acids there seems to be channel properties of the membrane.

Channeling Through Membranes

Both natural and artificial membranes contain a large number of discrete gating site (Hodgkin and Huxley, 1952) which open and close at random as revealed by fluctuation analyses (Stevens, 1977) and single channel recordings (Neher and Sakmann, 1976).

Channels undergo structural transitions between conducting and nonconducting states. A charge and ion transfer through the membrane and kinetics of this process seem to be common in bilayers, spheroids and natural membranes. It has been shown (Kennedy, 1967) that synthesized peptides of the sequence (Leu-Ser-Leu-Gly), having the helical structure, also form ion channels across the lipid bilayer membrane.

Nernst and Hodgkin-Huxley Formulas

Despite some criticisms (Habib and Bockris, 1982) the Nernst and Hodgkin-Huxley equations maintain broad acceptance as the explanation for the phenomenon of excitability. Their simplicity may be one reason for this.

The gradient of ions, both in concentration and in charge across an asymmetric membrane are the basic and sufficient factors explaining membrane excitability. The charge q of n ion moles is

$$q = z\ n\ F,$$

where

z : valency,
F : Faraday constant.

The differential of this value, in the case of the presence of more than one kind of ion, is

$$dq = \sum_{i=1}^{m} z_i\ F\ d\ n_i.$$

Diffusion of ions through a membrane also means simultaneous transfer of charge. This process, depending on the ratio of ion charge to membrane polarization and to its dielectric property, is governed by the diffusion force moving ions, and by an opposite electrophoretic force inhibiting ion movement.

If the diffusion of ions is inhibited, a state of equilibrium is reached. This state is described by the Nernst formula. It has been derived also from a very fundamental theoretical background. The chemical potential is

$$\mu = \mu^{o} + R\ T\ \ln\ p,$$

where

μ^{o} : reference chemical potential,
p : pressure,
R : gas constant.

In the case of dissolved substances there is a ratio of ions expressed by a_1 and a_2, the log of which when taken and multiplied by RT/zF gives the equilibrium potential P_{eq} as stated by the formula

$$P_{eq} = \frac{RT}{zF} \ln \frac{a_1}{a_2} .$$

It is worth emphasizing here the similarity of this formula with the formula for oxidation-reduction potential P_{or}:

$$P_{or} = E_o + \frac{RT}{zF} \ln \frac{A^{n-1}}{B^{m+1}} ,$$

where

E_o : standard electrochemical potential,

A^{n-1} : oxidized compound,

B^{m+1} : reduced compound.

The actual value of equilibrium potential across the membrane, estimated by the Nernst equation, in the living cell may be compensated and overcompensated by the metabolically driven potential. The intracellular versus extracellular proton concentration may be considered here as an equivalent ratio in the Nernst equation. The water environment of the cell, the role of water in electronic properties of peptides and proteins in membrane function seem to be evidence of the importance of protons in sustaining the membranes polarization and its dynamic properties during de- and repolarization (and generation of action potentials). In this case we can consider even a metabolically driven proton battery under ΔpH difference with an electromotive force E_p (Glaser, 1971)

$$E_p = 0.059 \log \Delta pH .$$

One unit of pH difference across the membrane is equivalent to 0.059 V of membrane potential. The resulting electrical potential across the membrane $\Delta\psi$ and the pH difference constitute the Mitchell proton motive force P_{mf}

$$P_{mf} = 0.059 \ (\Delta pH) + \Delta\psi .$$

Because of metabolism and its consequence upon the pH value, the actual voltage in the living cells exceeds P_{eq} value, and it follows that the membrane current I_m (Palti, 1971) is given by the following formula:

$$I_m = g_m (E_m - P_{eq}) ,$$

where

g_m : membrane conductance,
E_m : membrane potential,
P_{eq} : equilibrium potential.

When $E_m = P_{eq}$ the transmembrane current is zero. Such a situation takes place in artificial membranes after a certain time lapse, unless they are not charged.

COMPARATIVE DATA ON EXCITABLE MEMBRANES

Under strict conditions of preparation of bilayer membranes, and addition of KCl and Excitability-Inducing-Material they display electrical properties such as membrane potential, and spontaneous (Fig. 1), as well as current-induced electrical discharges (Fig. 2).

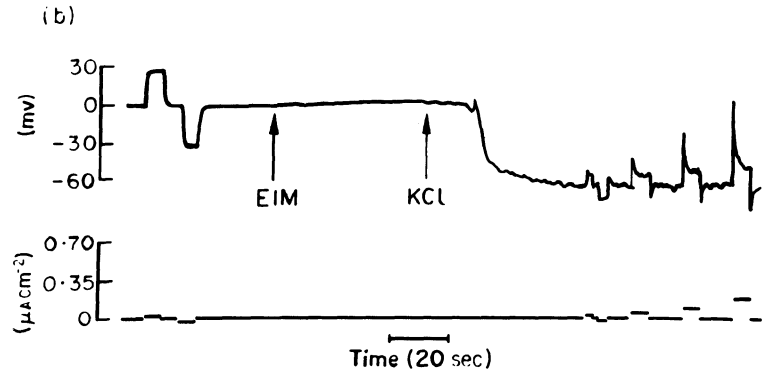

Fig. 1. Membrane and current-induced potentials of the bilayer lipid membrane (upper record) due to electrical stimulation (lower record) (Mueller and Rudin, 1968).

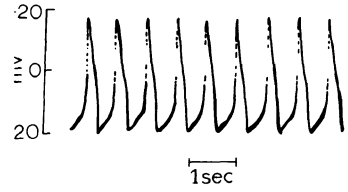

Fig. 2. Spontaneous electrical oscillations of the bilayer lipid membrane (Mueller and Rudin, 1968).

Very similar responses were observed in proteinoid spherules (Figs. 3-5). The difference was the presence in the first case of EIM and its absence in the proteinoid spherules.

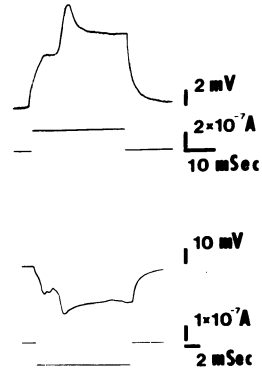

Fig. 3. Induced potentials (upper record) of the spheroidal membrane during electrical stimulation (lower record).

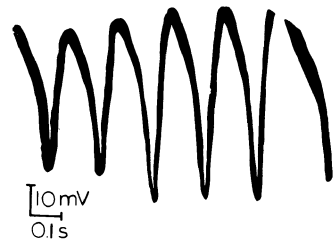

Fig. 4. Spontaneous electrical oscillation of the poly (Asp:Glu) proteinoid-lecithin artificial cell.

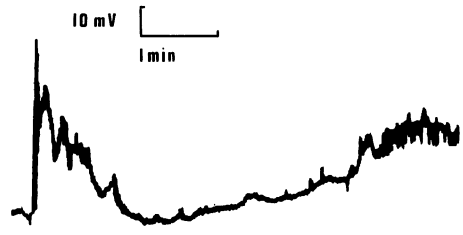

Fig. 5. Recovery of the membrane potential following mechanical injury of the proteinoid cell due to its microelectrode impalement.

There is also a high degree of similarity of the pattern of electrical discharges of the bilayer membrane and the spheric membrane of the proteinoid cell. Various patterns observed in proteinoid cells are different in regard to the shape and gradient of discharges (Figs. 6-8). Some highly resemble natural neuronal spiking (Fig. 6). The repeatable examples of electrical discharge display very similar patterning among spheres made of the same material (Figs. 6, 7). However, similar patterning of electrical discharges may be observed in spherules made of different polymers, although there are indications that patterning is connected with the chemical composition of microspheres. The resultant discharge pattern is conditioned by, at least, several factors such as membrane thickness, its ion permeability, etc. Because of this, the influence of composition of the cell

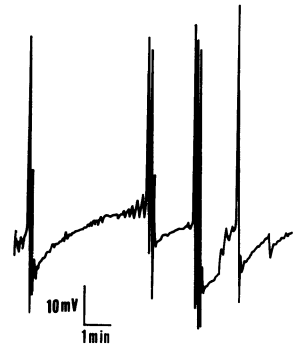

Fig. 6. Pattern of electrical discharges of the proteinoid cell made of the 2:2:1 proteinoid.

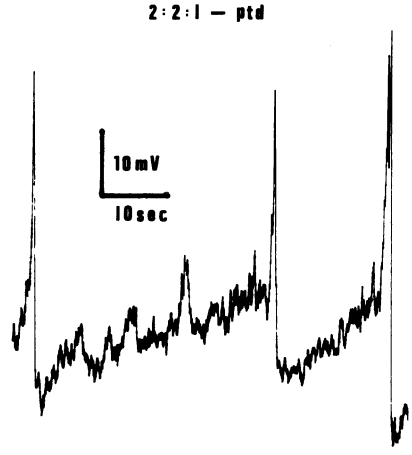

Fig. 7. Similar pattern of discharges of the proteinoid cell made of the 2:2:1 proteinoid several months later.

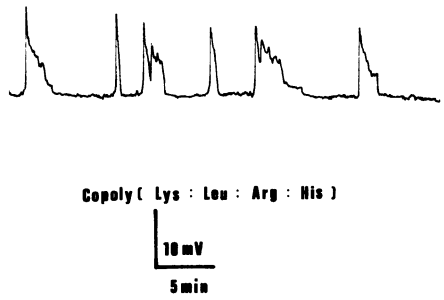

Fig. 8. Pattern of electrical discharges of the proteinoid cell made of copoly (Lys:Leu:Arg:His).

and, in particular, of the membrane cannot be simply related to its electrical properties.

The very characteristic feature of spheroidal membranes is their recovery, both of membrane polarization due to microelectrode impalement (Fig. 5) and external KCl treatment of the cell (Fig. 9). This implies an intrinsic homeostatic mechanism.

All the above seems to indicate not only a superficial but rather a deeper analogy between these artificial entities and natural excitable cells, including perhaps also the very mechanism underlying membrane polarization and its discharges.

An application (Stratten, 1982) of the Hodgkin-Huxley formalism in analysis of the proteinoid cell indicates common ionic mechanisms

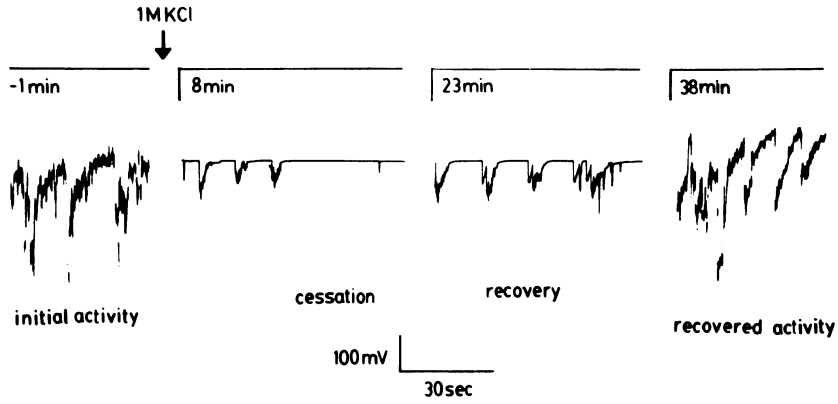

Fig. 9. Homeostatic recovery of the electrical discharges of the proteinoid cell (2:2:1-proteinoid) due to external KCl treatment of the cell.

of excitation in proteinoid and natural excitable cells. Evaluation of membrane potential E and its conductance g with intrasphere electrodes and current clamping with varying external potassium ion (K) concentrations clearly indicate a significant contribution of g(K). An induced reduction in E is accompanied by an increase in g(K) as is the case with nerve membrane, but it may also be accompanied by a decrease in g(Na) which is not manifested with nerve membrane.

There is more than a simple analogy between proteinoid excitable cells and neuronal or muscle cells. The similarities recognized so far are as follows:

RC membrane characteristics,
electrical stabilization by calcium ion,
transient depolarizing spiking,
spike recovery phase,
apparent homogeneity of channels,
negative inside resting potential.

Differences are rather secondary.

A comparison of some physical characteristics of artificial and natural membranes (Table 2) allows us to search for closer simistanding of construction and function of natural membranes and excitable cells.

In both cases of artificially made membranes (bilayer and spheriodal) there is, except light and ion concentration gradient across membrane, no energy source driving the electrical discharges. The absence of electrical discharges in darkness seems to be strong enough proof that illumination of the preparation even by day-light or white microscope-illumination light is the primary energy source of electrical discharges observed both in bilayer and spheroidal membranes. In regard to proteinoids, photoeffect is displayed (Przybylski and Fox, 1982). This property is due to the presence in the polymer of flavin and pterin chromophores (Heinz and Ried, 1981). These chromophores would be, hence, responsible for trapping of photons and electron release, whereas the membrane structure would be responsible for charge separation, i.e., holding the potential. As the consequence, periodic electrical discharges of the membrane would take place with a time constant determined by its resistance and capacitance. Additionally, in the case of the ionic gradient across the membrane, and due to the presence of the channel-like structure of the membrane, the ionic flow through the membrane would be influenced by changes in the membrane potential. These two factors seem to be responsible for the observed electrical phenomena in artificial membranes.

Table 2. Physical Characteristics of Bimolecular Lipid Membranes, Spheric and Natural Membranes

Membrane	Natural Membranes	BLM	Spheric Membranes
Thickness (Å)	40-130	60-90	10000
Potential difference (mV) (resting)	10-88	0-140	10-1000
Resistance (Ω-cm^2)	10^2-10^5	10^3-10^9	10^4-10^8
Capacitance (μF/cm^2)	0.5-1.3	0.3-1.3	0.7
"Excitability"	Observed	Observed	Observed
Ion selectivity and specificity	Observed	Observed	Observed
Excitation by light	Observed	Observed	Observed

CONCLUSION

In conclusion we can say that the experimental proteinoid protocell model, characterized by one or more proteinoid or proteinoid-lecithin membrane layers and by a proteinoid core with electrolyte, is also a model of an excitable cell.

The excitable artificial cell possesses many features characteristic of natural neuronal cells such as: membrane potential, all-or-none spontaneous and induced electrical discharges, hypo- and hyper-polarization type of membrane potential changes, asymmetric permeability, channeling phenomena, current-voltage characteristics with negative resistance, intra- and extra-cellular ionic influence upon the membrane potential including homeostatic recovery of discharge spiking and membrane potential.

The change of the membrane potential of the excitable cell is compatible with Hodgkin-Huxley equations. The light-sensitivity of the thermal amino acid polymer making up the artificial cell membrane. The flavin and pterin chromophores seem to be the energy trapping source which gives rise to the functions described.

ACKNOWLEDGMENTS

This study was also aided by grants from the National Aeronautics and Space Administration Grant No. NGR 10-007-008, and Mr. David Rose.

REFERENCES

Eley, D. D., Lockhart, N. C., and Richardson, C. N., 1979, J. Chem. Soc. Faraday Trans., 75:323.

Fox, S. W., McCauley, R. M., Montgomery, P. O'B., Fukushima, T., Harada, K., and Windsor, C. R., 1969, in: "Physical Principles of Biological Membranes," F. Snell, J. Wolken, G. J. Iversen, and J. Lam, eds., Gordon and Breach, New York.

Fox, S. W., Adachi, T., Stillwell, W., and Ishima, Y., 1978, in: "Light Transducing Membranes: Structure, Function, Evolution," D. W. Deamer, ed., Academic Press, New York.

Fox, S. W., and Nakashima, T., 1980, BioSystems, 12:155.

Glaser, R., 1971, "Einführung in die Biophysik," VEB G. Fischer Verlag, Jena.

Goldacre, R. J., 1958, in: "Surface Phenomena in Chemistry and Biology," J. F. Danielli, K. G. A. Pankhurst, and A. C. Riddiford, eds., Pergamon Press, London.

Habib, M. A., and Bockris, J. O'M., 1982, J. Bioelec., 1:289.

Heinz, B., and Ried, W., 1981, BioSystems, 14:33.

Hodgkin, A. L., and Huxley, A. F., 1952, J. Physiol. (London), 117:500.

Ishima, Y., and Fox, S. W., 1973, Abstract, Third Ann. Mtg. Soc. Neuroscience, 17.10:172.

Ishima, Y., Przybylski, A. T., and Fox, S. W., 1981, BioSystems, 13:243.

Kennedy, S. J., Roeske, R. W., Freeman, A. R., and Besch, Jr., H. R., 1977, Science, 196:1341.

Lewis, T. J., 1982, Phys. Med. Biol., 27:335.

Mueller, P., and Rudin, D. O., 1968, J. Theor. Biol., 18:222.

Neher, E., and Sakmann, B., 1976, Nature, 260:779.

Palti, Y., 1971, in: "Biophysics and Physiology of Excitable Membranes," W. J. Adelman, Jr., ed., p. 168, Van Nostrand Reinhold Co., New York.

Przybylski, A. T., Stratten, W. P., Syren, R. M., and Fox, S. W., 1982, Naturwiss., 69:561.

Przybylski, A. T., and Fox, S. W., 1982, in: "Alternative Energy Sources," Vol. 3, T. N. Vezirolgu, ed., Ann Arbor Science, Ann Arbor.

Rosen, D., and Sutton, A. M., 1968, Biochim. Biophys. Acta, 163: 226.

Rutten, M. G., 1971, "Origin of Life" Elsevier Press, Amsterdam.

Stevens, C. F., 1977, Nature, 270:391.

Stratten, W. P., 1982, Abstracts Soc. Neuroscience Ann. Mtg., Minneapolis, 66.12.

Szent-Györgyi, A., 1941, Nature, 148:157.

Takenaka, T., Inoue, I., Ishima, Y., and Horie, H., 1971, Proc. Japan Acad., 47:554.

Tien, H. T., 1974, "Bilayer Lipid Membranes," Marcel Dekker, New York.

White, S. H., 1970, Biophys. J., 10:1127.