INTRODUCTION

The term bio-excitation generates many mental pictures ranging from the machinations of the brain, responsiveness to environmental stimuli, facilitated efferent motor responses, electrical discharges, ionic gradients, membrane structure, etc. At the cellular level, those familiar with excitation immediately retrieve one model or another upon which they have depended for mental imagery. Some begin with a precise phospholipid bilayer membrane peppered with protein gates or carriers with precision architecture. Others also consider the biochemical and energy transfer processes associated with active transport, and maintenance of membrane anisotropy. The eons of natural selection which have allowed the development of such sophisticated electrochemical processes concerns yet others. And there are many primarily concerned with the process by which external chemical, electromagnetic, or mechanical energies are converted into excitation. But what becomes of all of these images if it is suddenly found that simple heating and subsequent hydration of amino acid mixtures results in spherical structures manifesting all fundamental characteristics of excitation? There is no need for biological evolution. There is no need for phospholipids. There is no need for nucleotide control of protein precision. There is no need for ATP or other sophisticated metabolic processes. There is no apparent afferent-efferent link approximating in vivo excitation processes. There is no longer any intimate relationship to intercellular communications, or electrical induction of sophisticated intracellular enzymatic processes. All of these facts have profound impact upon our conceptualization of the process of excitation.
It is the purpose of this chapter to compare some of the popular imagery of excitation with the realities demonstrated by the discovery of excitation in proteinoid protocells. This will hopefully provide incentives to some investigators of excitation to approach its unanswered problems with a fresh perspective.

MODELS FOR MEMBRANE EXCITATION

Since the publication of the Hodgkin and Huxley theory (1952), based upon the concept of "gated" ionic flow, it has dominated the field of membrane excitation. This has been the case in spite of the fact that it gives little consideration to the physicochemical nature of the membrane. This theory has been followed by others portraying the process as dipole flip-flops (Wei, 1972), simple induction (Ling, 1982), ionic energy barrier transitions (Hille, 1975), membrane ion exchange (Tasaki, 1968), acetylcholine induced gating (Nachmansohn, 1970), or even hydrostatic induction of capillary action (Teorell, 1962). This chapter's limited review of the Hodgkin-Hurley, Tasaki, and Nachmansohn models and the lipid bilayer work of Mueller and Rudin will hopefully provide adequate example to indicate the potential impact of the protocell findings.

Hodgkin and Huxley

Using the results of voltage-clamp experiments on squid giant axon, Hodgkin and Huxley (1952) began with a mathematically simplifying assumption that the current flow through the membrane could be separated, i.e., the current of each ion could be considered to be flowing through its own selective path. The total current was the sum of that flowing through all of the parallel paths. Equations were then formulated describing the relationship of the ion conductance to the variables of time and membrane potential. The sum of potassium and sodium ionic flow explain most membrane responses. Of the many conductance-time empirical expressions which could be chosen to fit the sigmoid rise in potassium conductance or the transient rise-fall of sodium conductance, Hodgkin and Huxley (1952) used expressions portraying multi-particle movements. The concept of "movement" and membrane semipermeability allows the application of the Nernst equation and the assessment of the membrane potential contribution of each ion. The model fits the observed phenomenon in a broad set of conditions and the "gate" forms a simple mental picture. The combination of its fit, its simplicity, and its precedence have kept this model on the forefront of membrane excitation studies.

Tasaki

The Tasaki model (Tasaki, 1968; Singer and Tasaki, 1968) includes considerations of the physicochemical properties of the
membrane--at least to the limited extent to which they are known. Using varied internal and external ionic milieu, membrane function can be explained by steric alterations of macromolecular structure induced by univalent/divalent cation interactions. The membrane current is not via simple flow-through "gates" but rather a membrane transfer of ions due to sequential exchange between the divalent and the univalent cations. The macromolecular cation "carriers" have two stable conformations. In the resting state, the macromolecules are occupied primarily by divalent cations which are not readily displaced as long as the membrane potential maintains its inside-negative polarity. Activation results from displacement of the divalent cation by univalent cations. In the "active" state the univalent current efflux soon decreases (sodium vs. potassium) and upon diminution the divalent cations once again manifest their stronger affinity for the membrane's external anionic sites (Singer and Tasaki, 1968). Although some claim that the Tasaki model is not significantly different from Hodgkin-Huxley's (cf. Mueller and Rudin, 1969a), there exist several phenomenon which cannot be explained by the Hodgkin-Huxley model, but seem to be adequately explained by the Tasaki model: (1) Effects of dilution of external univalent cations can be mimicked by concentration of external divalent cations. (2) The resting potential resulting from external potassium ion dilution does not obey the Nernst equation (its elimination will not alter the resting potential). (3) An action potential spike can still be manifest when internal and external sodium concentrations are equal. (4) Tetrodotoxin, a presumed sodium "gate" blocker, blocks action potential and inward current in sodium-free media (Singer and Tasaki, 1968).

Fundamental to most of the disagreement between the Tasaki and Hodgkin-Huxley models is the indication that there exists no ionic specificity, i.e., to sodium and potassium. Rather, a lyotropic sequence of favorability is supported. The depolarizing cations such as potassium can actually be substituted for sodium in its hyperpolarizing role. In fact, the external univalent cation can be eliminated altogether, given the proper balance of lyotropic substitutes. Even the anionic lyotropic sequence influences excitability, fluoride being the most favored, indicating a competition between the membrane and media anions. The cation fixation to membrane "carriers" can account for these lyotropic sequences of the internal anions, but it cannot be explained by the Hodgkin-Huxley model (Singer and Tasaki, 1968).

Nachmansohn

The model of Nachmansohn illustrates membrane excitation as a sequence of events beginning with a stimulus induced release of acetylcholine (ACh) from its membrane-bound loci. The "free" ACh quickly associates with a neighboring protein ACh receptor, inducing a conformational change in the protein. The conformational
change results in the release of calcium ions which interact with
other membrane macromolecules, altering the membrane's ion perme­
ability. Membrane associated acetylcholinesterase quickly hydolyzes
the ACh and the processes reverse, reestablishing the ionic barrier
(Nachmansohn, 1970). The membrane potential is represented by the
sum of active and passive transport. The metabolic energy exchanging
enzymes essential for the active processes are also thought to be an
intrinsic component of the excitation complex. This model is based
largely upon the research on fish electric organs, but Nachmansohn
refers to it as the "unified concept of the role of ACh." He claims
that "the elementary processes that change ion permeability are
essentially the same in the axonal, in the nerve terminal, and in
the postsynaptic membrane" (Nachmansohn and Neumann, 1974). This
claim is supported by a variety of circumstantial evidences such as
the finding of ACh, and acetylcholinesterase in a wide variety of
excitable tissue; however, there exists considerable evidence
against this view (Koelle, 1966). This model represents a growing
notion that the excitation process is a result of interaction of a
sophisticated set of highly evolved interacting enzymatic components,
implying that the process of excitation may be a newcomer in the
evolutionary scheme (Neumann et al., 1973). If one is to assess
the process of membrane excitation, from an evolutionary perspective,
this model should be included even though it may only apply to a
specialized tissue.

Black Lipid Membranes

Although not proposing a unique model per se, Mueller and Rudin
(1968, 1969a,b) have made significant contribution to our under­
standing of membranes by their work using bimolecular membranes
formed from cellular lipids. These membranes are often referred to
as black lipid membranes (BLM) due to their failure to reflect visible
light. The properties of the artificial membranes in terms of thick­
ness, water permeability, surface tension and passive electrical
characteristics are very similar to those of cell membrane (Mueller
and Rudin, 1969b). The BLM electrical properties are all passive
and linear in nature until supplements are added. Those supplements
which convey alteration in electrical and/or chemical affinity char­
acteristics are referred to by Mueller and Rudin as translocators.
Some of the translocators impart simple increases in conductances
which display Ohmic linearity, while others display non-linear
potential-conductance relationships resulting in activity similar
to action potentials (Mueller and Rudin, 1969a).

Excitability inducing material, EIM, a cytolytic bacterial
endotoxin, was the first compound found to induce a voltage depen­
dent conductance in the lipid membranes. The EIM translocated
species are cations and the electrical dynamics are similar to those
of potassium in nerve (Mueller and Rudin, 1969b). The addition of
protamines or polyamines to the EIM membrane changes the translocated
species to anions. The proper titering of EIM and protamines will display bimodal translocation, cationic and anionic, which manifests action potentials exactly like those of the alga Nitella, and similar to nerve, albeit it is an anion-cation selectivity instead of sodium-potassium. Monazomycin another voltage dependent translocator possesses bimodal translocation properties, carrying hydrogen ions in one conformation and potassium or sodium ions in the other. This alleviates the necessity for two separate channels (Mueller and Rudin, 1969a).

STRUCTURE OF EXCITABLE MEMBRANE

Protein-Lipid Cooperation

The general view of cell membrane structure has changed little over the past nine decades. In 1895 Overton (cited in Troshin, 1966) described the membrane as a matrix of nonpolar lipids supporting arrays of localized polar carriers or ion channels. A later refinement, the Danielli-Davson model, is a phospholipid bilayer "unit membrane" to which proteins are attached (Nachmansohn, 1970). The model represents the lipid component as a passive structural barrier whose functional loci are various adsorbates, most likely proteins. It is these proteins which impart the properties of semi-permeability, excitation, metabolism, transport, and enzyme activity to the membrane (Mueller and Rudin, 1969a). More precisely, since the proteins themselves are modified by the forces of the lipid-water interface, the membrane appears to be comprised of functional lipoprotein complexes with the lipid-modified protein structure serving as the functional core of each complex (Nachmansohn, 1970). The view that these functional complexes are "fixed" within the lipid matrix of the membranes is not supported by recent work on artificial membranes (Mueller and Rudin, 1969a).

Macrostructure

Numerous studies have indicated asymmetric membrane structure, attributable to the protein components. Evidence to support this includes (1) the external application of proteases to squid giant axon has no effect but internal application is damaging, (2) the membrane exterior is cation sensitive while the interior is not, (3) the interior is anion sensitive while the exterior is not, and (4) differences exist as to the interior vs. exterior sensitivity to alteration of heavy metal, organic ion, or hydrogen ion concentrations (Singer and Tasaki, 1968).

Lipids

The intra-membrane arrangement of lipid molecules has not been totally resolved. The formation of the lipid bilayer is energetically strongly favored; however, the work with artificial membranes
indicates that there is no energetic preference between the formation of bilayer spheres, spheres of many bilayers, small micelles, or even an extended smectic form. Mueller and Rudin (1969a) have found, however, that the multiple layering and smectic phases are broken up by the addition of proteins, leaving a likelihood of only the bilayer form of micellar structures as being the biological conformation. Their arrangement in a bilayer is supported by electron microscopy, X-ray electron density, X-ray diffraction, birefringence, and thermal phase transition. BLM are of known lamellar conformation; and their properties of membrane resistance, lipid/water solubility ratio, protein association, excitability, and scanning calorimetry are found to be the same as those of natural membrane. However, Sjoestrand and Barajas (1968) using techniques designed to preserve protein structure, have found micellar structures in electron micrographs of the membrane.

BLM have high electrical impedance, five to seven orders of magnitude higher than cell membranes (Mueller and Rudin, 1969b). It is only with the addition of proteins or other conductive adsorbates that the membrane displays electrical conductance of the magnitude displayed by biological membranes. Little is known about the degree to which the lipids are involved in the facilitation of the adsorbate conduction increase. There seems to be no general dependence of the adsorbates on the lipids, but with artificial membranes one adsorbate, the polyenes, require cholesterol to be active (Mueller and Rudin, 1968). In at least one case the function of the adsorbate changes with a change in the lipid structure. Dipicrylamine transfers dynamically from one surface to the other of one membrane type and acts as the charge carrier. In another lipid (lecithin) the dipicrylamine acts as an ion carrier for potassium or hydrogen ions (Mueller and Rudin, 1969a).

Protein

Ions are accepted to be the carriers of membrane currents. The mechanism accounting for the rapid dynamics of action potentials is the primary riddle for the electrophysiologists today. The answer to the riddle seems to lie hidden with other protein properties (Nachmansohn, 1970).

The great diversity of membrane function, specificity, and efficiency is attributable more to the proteins than to the phospholipid composition. The precision stereo selectivity, and the effect of sulfhydryl-blocking and disulfide-reducing agents on excitation receptors support the notion of their protein identity (Karlin and Bartels, 1966). There are also indications that cell membranes are highly ordered dynamic structures, the architecture of which facilitates the membrane's intrinsic activities (Neumann et al., 1973). One underlying question of many studies is that of how much "order" is essential for any given membrane function. Complexity appears
in most fundamental models, e.g., Hodgkin and Huxley were able to quantitatively fit their analytical data only after assuming that a fixed multiple of charges were associated with each equilibrium state (Mueller and Rudin, 1969a). Kennedy et al. (1977) have formed ionic channels in membranes by the application of simple synthetic polypeptides; however, these polypeptides were modeled after biological structures known to influence membrane permeability. Even these simple polypeptides were found to induce permeability changes only upon hexameric formation.

**Protein Complexes**

The more advanced models of functional membranes are even more replete with the notion that complex organized protein structures are essential to excitation. Macromolecules and their multiple internal or external linkages are essential to the model portrayed by Tasaki (1968). The model of excitation via field-induced conformational changes in biopolymers causing highly selective alteration in ion permeability, alludes to highly specialized macromolecular mediation (Nachmansohn and Neumann, 1974). The occurrence of extrinsically gated translocators (possibly the fundamental mechanism of chemoreceptors) implies a heterotropic allosteric system (Mueller and Rudin, 1969a). Nachmansohn (1970) claims that all sites of excitation are complex membrane structures including a multiprotein receptor, a deactivating acetylcholinesterase, and other macromolecular components. The Katchalsky model (Neumann et al., 1973) also views excitation units as being multiple protein gateway complexes possessing a structure and charge array which is dynamically altered with change in field potentials.

**Ions and Cations**

Ions also have structural implications via their membrane interactions. The work of Tasaki with squid giant axon links divalent cations with the manifestation of excitability. Ionic affinities indicate that the external surface of biomembrane possesses an excess of intrinsic negative charge (carboxylate) and the inner surface shows an excess of positive charge (phosphate) (Singer and Tasaki, 1968). External divalent cations, are suspected of contributing to the maintenance of membrane structure by bonding adjacent negative macromolecule side groups (Schellman and Schellman, 1964). This is supported by the finding that when the external medium is limited to univalent cations, the axons become inexacitable. The cation exchange process between divalent and univalent cations is the key to the function of the "two-stable-state" membrane model of Tasaki (1968).

The most functionally relevant divalent cation seems to be calcium. The monitoring of osmotic coefficient by Katchalsky (1964) indicates the 99% of the calcium is bound to the resting
biomembrane. This is in agreement with the prediction of Tasaki, who contends that excitation would accompany the release of the calcium (Singer and Tasaki, 1968). The notion of protein conformational changes upon calcium release is supported by the known potency of calcium in inducing such changes. This process is especially dramatic on surfaces possessing abundant negative charge (Nachmansohn and Neumann, 1974).

Protein Basics

In spite of the many complex models, few ask the basic question: "What are the minimum requirements for membrane excitation?" It must be remembered that most proteins are ampholytes with the N-terminal being positive and the C-terminal negative (Singer and Tasaki, 1968). This common property of all amino acids added to the varied polar and apolar properties of the various R-groups leads one to believe that there are many likely protein conformations able to (1) change conformation within an electrical field, (2) carry ions, and (3) possess sufficient lipophilicity to function in association with lipids. The sum of these attributes makes the possessor a likely candidate as a translocator of non-lipid-soluble inorganic ions which are normally restricted to the aqueous phase (Mueller and Rudin, 1969a).

Protein Membrane

Since the role of lipids appears to be that of a passive barrier to charge flow, and since some proteins also possess strong apolar characteristics, it might be guessed that membranes could also be formed from proteins alone. Under select conditions such have been formed and have dimensions similar to lipid bilayers; however, they are found to be "leaky" in relation to the lipids (Mueller and Rudin, 1969a; Anderson et al., 1953; Fox and Dose, 1977).

PROTEINIODS, PROTEINOID MEMBRANES, AND PROTOCELLS

Proteinoids

Proteinoids are synthetic copolyamino acids formed by heating various mixtures of amino acids. The polymers are self-ordering (Fox and Dose, 1977). This is likely due to the "ordering" propensity of the precursor amino acids (Fox, 1978). The ordering tendency is carried beyond the intraproteinoid level to ordering for populations of proteinoid molecules to form membranes and homogeneous sphere populations. This macro-ordering is an indication of repetition of order from one proteinoid or proteinoid array to the next (Fox and Dose, 1977). In addition to the self-ordering, these polymers have many characteristics fundamental to life processes, including the many structural and functional aspects outlined in the following paragraphs.
Kept aseptic, the proteinoids are indefinitely stable within a broad range of pH, temperature, and hydration conditions (Fox and Dose, 1977).

Proteinoids abundant in hydrocarbon-rich amino acids display lipid-like properties, and are among those which combine most readily with lecithin and other lipids. It is these proteinoids which have the highest probability of displaying properties of excitation (Ishima and Fox, 1973). Electrical excitation requires the ambivalence of electrical insulation and conductivity. Since electrical excitation is manifest in some proteinoid complexes lacking any phospholipid, the proteinoids must possess both properties (Przybylski et al., 1982). The presence of both hydrophobic and amphophilic regions along the surface of the polymers allows such to be possible. The most strongly conductive products found amongst proteinoids and their fractions are also oil-soluble. The balance between these two properties is likely to be essential to the manifestation of excitability (Grote et al., 1978).

A broad array of catalytic activities have been discovered for the proteinoids. These include various forms of hydrolysis, decarboxylation, amination, oxidoreductions, photoactivated decarboxylation, hormonal interactions, and synthesis of internucleotide and peptide bonds with ATP (Fox et al., 1978; Fox and Dose, 1977).

The addition of either proteinoid polymers or polymer fractions increases the conductance of chloroform solutions indicating these thermal products to be current carriers (Grote et al., 1978). These findings are not unexpected since thermal copolyamino acids have ESR densities of $10^{18}$/g (Bone et al., 1978) allowing visualization of the proteinoids as shuttling electrons or holes. Those proteinoids or fractions which are the most effective in solutions are also the most effective in enhancing phospholipid membrane conductance. This circumstantial association may imply that the current of the thin layer membrane may be "carrier-mediated" as it is in the bulk solvent, but does not eliminate the possibility of other ionophoric or porous modalities (Grote et al., 1978).

In summary, the proteinoids appear to be able to stand as a "one man band" having diverse properties and functional capacities of several chemical classes including those of lipids, proteins, and even the nucleic acids. The possession of this diversity may obviate the need for communion of many complex molecular classes in the manifestation of primitive life function (Przybylski et al., 1982).

**Proteinoid Membranes and Protocells**

**Structure.** Proteinoids form membranes, either of the spontaneous spherical form upon hydration (Fox and Dose, 1977), or black
bilayers using special techniques similar to those of Mueller and Rudin (1969b). Electron micrographs show the proteinoid sphere membrane to be a double layer, but the membranes of many are thicker than the BLM. These proteinoid membranes are "leaky" as were the protein membranes mentioned previously, but possess many properties of "tight" membranes (Przybryski et al., 1982; Fox and Dose, 1977).

Many proteinoids have lipid-like properties, these properties being attributed to non-polar amino acid side chains (Lehninger, 1975). It is the proteinoids which have these lipid-like properties which form membrane and spheres (p-protocells) (Przybylski et al., 1982). Adding lecithin during hydration results in the formation of electrically excitable proteinoid-lecithin spheres (p-l-protocells). The apolar side chains seem to be important, possibly for an essential complexing with the lecithin in the sphere formation. Three amino acids with apolar side chains, leucine, proline, and threonine, are of special interest (Ishima et al., 1981).

The coordination of ions by the translocator polypeptide complex of Kennedy et al. (1977) is thought to mediated by hydroxyl groups of serine and to be essential to electrical activity. Comparatively, it is possible that threonine hydroxyls are the "ion coordinators" in the protocell membrane since they seem essential to excitability (Ishima et al., 1981).

Properties. The p-protocells maintain the several properties just outlined for the proteinoids alone, but include new properties related to its various attributes of membranicity, and ordered macrostructure (Przybylski et al., 1982; Fox and Dose, 1977).

In addition to the attributes of stability attributed to the proteinoids alone, the protocells maintain remarkable durability. Visual and electrical examination reveal tolerance to extremes of pH (Snyder and Fox, 1975), temperature and dehydration (Fox and Dose, 1977; Ishima et al., 1981). Mechanical resilience is demonstrated in the self-sealing of black membranes following puncture, a property matching that of lipid bilayers or biological membranes (Fox et al., 1978).

The incorporation of the proteinoid catalytic activity into the protocells is indicated by the glucose catalysis (Fox and Krampitz, 1964), peroxidase, and phosphatase activities of the spheres (Hsu and Fox, 1976). The spheres also have the ability to synthesize peptides in water (Nakashima and Fox, 1980).

The spheres manifest junction formation and visibly participate in intercellular activities (Hsu et al., 1971).

The protocells manifest selective permeability (Fox, 1969) and the osmotic properties which accompany this membrane property
(Fox et al., 1978). Being selective, they are effective in maintaining anisotropy for long periods of time (Ishima et al., 1981).

**PROTOCELL EXCITATION**

**Introduction**

A number of proteinoid and proteinoid-lecithin combinations manifest nerve-like electrical properties (Ishima and Fox, 1973; Ishima et al., 1981; Przybylski et al., 1982; Stratten, 1982). The electrical properties vary with the identity of the proteinoid. Four of one hundred proteinoids tested with lecithin were found to manifest significant characteristics of excitability under given conditions. Expectedly, variables other that proteinoid identity influence excitation. Of the many solutions evaluated, Ishima et al. (1981) found artificial pond water to be optimum. Subsequent work by Przybylski et al. (1982) with p-protocells indicates transmembrane potassium ionic disequilibrium as a minimum requirement for membrane excitability. Spiking can be induced in active protocells by shifts in external ionic concentrations. For p-l-protocells the external medium must be adjusted to levels hypoosmolar to the internal medium to induce the emergence of a thin, transparent, spherical membrane from the p-l crust; there are increases in membrane stability with incubation; and incubation periods for p-l protocells of 2 hours to 2 days increase the yields of units manifesting excitability. Increased concentration of calcium ion concentrations in the external fluid decreases the spontaneity of the membrane electrical activity as it does for neurons (Ishima et al., 1981).

**Proteinoid-Lecithin Protocells**

The electrical properties of the p-l protocells seem to be provided by a conductive component of proteinoid and an electrically insulating component of phospholipid. This is equivalent to the neuronal component properties (Nachmansohn and Neumann, 1974). The p-l-protocells manifest five potential types: (1) a steady-state resting membrane potential, lying between -20 and -70 mV with the mean at -44 mV, (2) a flip-flop activity similar to that expected in the "two-stable-state" hypothesis of Tasaki (1968), (3) singular spikes resembling neuron action potentials, (4) bursts of spikes, or (5) miniature activity at the flopped (hyperpolarized) phase, comparable to miniature end-plate potentials of neuronal soma. Further data has been acquired by alteration of external potassium concentrations and application of transmembrane square wave current pulses. Elevation of potassium results in membrane depolarization. The current pulses induced Ohmic changes in the membrane potential at low currents, but spiking at the higher currents (Ishima et al., 1981).
Proteinoid-only Protocells

The membrane potentials and excitation manifest in p-l-proto­
cells are also manifest in p-protocells. Although the latter mani­
fest higher conductance and greater instability, only quantitative
 differences in electrical properties are indicated between the two
(Przybylski et al., 1982).

Application of Hodgkin-Huxley Model

An evaluation of protocell electrical activities by application
of the Hodgkin-Huxley model reveal many similarities in biological
systems.

The resting membrane potential is determined by the membrane
conductance to potassium and sodium. The sodium conductance is
higher than that of neurons, but potassium plays the prime role
(Stratten, 1982).

The elevation of external potassium ion reduces the membrane
potential and increases potassium conductance (Ishima et al., 1981).
Application of the Nernst equation indicates that the potassium con­
ductance increases are associated with an opposing decrease in
sodium conductance (Stratten, 1982). The increase in potassium con­
ductivity is similar to that found with nerve axon membrane when
the membrane potential is voltage clamped at lower membrane poten­
tials; however, the biological tissue manifests no steady state
decrease in the conductance of sodium as does this preparation.

Spike generation appears to result from a shift in one or more
of the ionic conductances. Calculations indicate an increase in
sodium permeability during the spike rising phase, the increase
being disproportionate to that of potassium. However, the increase
in sodium conductance may be less than twofold, quite miniscule in
comparison to the 500-fold increase seen in nerve cells. The spike
shift in sodium conductance is comparable to that of nerve axon
membrane in its brevity (approximately 2 msec) (Stratten, 1982).
Application of successful dynamic translocators such as EIM have
all been quite slow in comparison to bioresponses (Mueller and
Rudin, 1969a).

The RC constant of the spike recovery phase indicates total
recovery of the sodium and potassium conductances to the resting
levels. The repolarization is attributable primarily to potassium
conductance, as is the case with nerve cell spike recovery
(Stratten, 1982).
Summary

The following are some of the similarities and differences between the protocell and the excitable nerve cell. It might be noted that most of the similarities are qualitative, whereas most of the differences are quantitative. Protocell-neuron similarities include: (1) RC time constants, (2) spiking depolarization attributable to increased sodium conductance, (3) spike duration, (4) spike recovery attributable to potassium conductance, (5) negative resting potential attributable to sodium and potassium conductance, and (6) stabilization by external calcium ions. Protocell-neuron differences include: (1) variable resting membrane potential, (2) spiking thresholds, (3) greater RC instability, (4) much less change in sodium conductance with spiking, (5) shifting conductance with steady-state depolarization, (6) higher resting sodium conductance, and (7) the membrane environment in which spiking occurs (Ishima et al., 1981; Stratten, 1982).

ENERGETICS AND EXCITATION

Metabolism

Anisotropism is essential for electrical excitation. The second law of thermodynamics demands a continual degradation of this anisotropy; therefore, it is assumed that this degradation must be compensated by endergonic processes. This need has evolved into a contest between those who feel the membrane potential is coupled with metabolism (Nachmansohn and Neumann, 1974; Hill, 1958) and others who contend the metabolic process is not necessary to the presence of a membrane potential (Troshin 1966; Tasaki, 1968). The evidence supporting either view is largely circumstantial.

Metabolism-Coupled Excitation

The metabolism-coupled view is supported by the high levels of heat production and absorption accompanying electrical activity, implying a tight association with chemical energetics processes, i.e., metabolism (Nachmansohn and Neumann, 1974; Hill, 1958). Nachmansohn (1970) has also found the energy production and absorption, "metabolism", of the Electrophorus to be low except in the membrane.

Non-Coupled Excitation

The non-coupled view is exemplified in the work of Tasaki (1968) who, with others, has shown the squid axon to be capable of conducting impulses for days with most of the cellular axoplasm removed. Although some of the characteristics may be due to residual internal and external components, since soluble metabolites are
gone in a few minutes, metabolism, if any, must be restricted to within the membrane. These findings in combination with those of BLM research implies that the metabolism, if coupled to excitation, must be catalyzed by the very substances which are the membrane translocators. The artificial membrane studies indicate only dissipative energy transduction mechanisms, alleviating such a necessity (Mueller and Rudin, 1969a). Tasaki (1968), further, finds that the exothermia associated with the initiation of an action potential is adequately explained by the accompanying displacement of divalent by monovalent cations. Similarly, the repolarization is endothermic due to a reverse in this cation exchange.

**Energy Conversions**

More generalized research on membrane energetics indicate biological membranes to be capable of interconverting between bonding, redox, and ion gradient energy forms. Of the six conversions most biomembranes possess only a few. The protocell excitation dramatically illustrates, at the very least, an example of STATE $I > STATE II > STATE I$. Such a sequence demands use of at least two conversions, one exergonic and one endergonic. The energy source, i.e., bond, redox, or ion gradient, is not yet obvious.

The work with BLM and certain translocators, e.g., monazomycin, reveals dynamic ionic affinities coupled with a sequential double gating effect. Jardetsky (1966) indicates such a combination as essential to pumping action. This sequential-valve/ion-dissociation pump could presumably provide the endergonic service of maintaining membrane anisotropy by coupling with a net exergonic "excitation" while incurring only minimal secondary energy losses. Redox agents could presumably act to provide electron energy to these pumps as easily as to the postulated oxidative phosphorylation processes. Mueller and Rudin (1969a) have gone so far as to say: "Many properties of the gated translocators resemble those attributed to active ion transport mechanisms. . . ."

Membranes may be involved in energy trapping and conversions including various phosphorylations with the polyphosphates, the pyrophosphates, or even ATP (Lipmann, 1965). Such has been demonstrated in the synthesis of amino acid polymers by ATP-protocell combinations (Nakashima and Fox, 1980).

**Photo-Excitation**

Photosensitivity has been found to induce "spontaneous" electrical activity of some protocells (Przybylski et al., 1982). Chromophores (pteridines and flavins) formed as byproducts of the formation of the copolyamino acid polymers or chromatic proteinoids themselves may act as the pigments channeling the photon energy toward enhancing membrane excitability (Heinz et al., 1979).
Although there is not yet a clear link between this photon-chemical energy coupling and the energy coupling of excitation, the enhancement of excitation with illumination arouses stimulating ideas associating the two.

Similar photon influences have been noted in biological membranes following addition of pigments. Singer and Tasaki (1968) found addition of pigments to allow light-mediated induction of excitation. In addition, electroplax, following addition of the pigment p-phenylazophenyl-trimethylammonium chloride, demonstrate wavelength sensitive potential changes (Nachmansohn, 1970). Although the latter may be associated with shifts in the cis-trans equilibrium, the mechanism of photon-excitation coupling is far from clear.

EVOLUTION OF MEMBRANE AND EXCITATION

The various enzymatic, permeability, and excitation properties of membrane are more likely physicochemical properties of protein than phospholipid (cf. Nachmansohn, 1970). The numerous roles of accretion, enzymatics, selective permeability, dynamic "gating", active transport, and maintenance of anisotropy are all functions which are presumably filled by proteins and have been shown to be filled by proteinoids. Only the last, a passive process, can be accomplished by the lipids. In spite of this, there are some investigators who insist that since "lipids are required for the formation of the only known examples of cellular membranes" it follows that lipid structures preceded those of protein, i.e., proteins are "extrinsic" additions (Mueller and Rudin, 1969a). It seems far more likely that a system first acquires the multiple selective advantages of molecular order and ordering via a primal proteinoid composition and only subsequently eases its burden of anisotropy maintenance by acquisition of the lipid. The argument that "the protein membranes are more permeable . . . therefore are not able to provide an efficient barrier structure" (Mueller and Rudin, 1969a) is considerably weakened by the myriad of stable proteinoid protocell types displaying many life-essential characteristics (Fox and Dose, 1977).

The argument "lipid first" is as untenable as the "DNA first" notion, which has been peddled as "truth" in most biology texts for the past two decades. In fact proteinoids and their bilayer protocells can be synthesized without DNA (Fox and Dose, 1977), but the opposite, i.e., DNA without protein, has never been shown. Similarly, the proteinoid membranes manifest essential characteristics of membranicity sans lipid, but the opposite has never been documented. The Miller-Urey-like experimental synthesis of hydrocarbons and long-chain fatty acids by Wilson (1962) and Allen and Ponnampерuma (1967) respectfully, does not alter the intrinsic lipid inadequacies. It simply implies that lipids may have been available in early stages of evolution to associate with and stabilize the protein structures.
There indeed would have been a selective advantage of those proteinoid-lipid protocells which had struck the optimal balance between the advantages and disadvantages of leakiness. The maintenance of some leakiness has advantages (Kuhn, 1976) including optimizing acquisition of molecular intermediates for structure, metabolism, etc., until the protocells acquire abilities to synthesize these from smaller components. Conversely, too great a leakiness would result in osmotic, anisotropy, and molecular intermediate retention problems (Mueller and Rudin, 1969a). One might conjecture that the "best of all worlds" would be the ability to control the moments of leakiness, in particular if they could be timed to correlate with the availability of beneficial substances. This may, indeed, be the earliest of selective advantages provided by the process we now call excitation. In summary, a likely sequence on the evolutionary path begins with proteinoid spheres which are eventually displaced by an advantageous proteinoid-lipid combination.

The finding that artificial fossilization of proteinoid spheres results in structures indistinguishable from fossilized algae (Barghoorn and Tyler, 1965) and that some active spheres are stable at high pH similar to that of the conjectured primordial alkaline ocean (Snyder and Fox, 1975) stand as circumstantial reinforcement to the "proteinoid first" notion.

Some of the current models of bioexcitation declare highly evolved molecular complexes (Neumann et al., 1973; Nachmansohn and Neumann, 1974), to be essential to the manifestation of excitation. The fact that such is the case for some types of excitation is likely, but arguments such as "it would be difficult to reason that the enzyme . . . is essential for electrical activity in some, but not others" (Nachmansohn, 1970) are inadequate. In fact, the manifestation of excitation in BLM by addition of some simple translocators seems to preclude such complexity, especially any essential linkage to cellular metabolism. However, the need for highly evolved structures for excitation was not totally obviated by this work, since the effective translocators have primarily been biological macromolecules with clear membrane-linked function which is highly selective and probably a result of natural selection. The most effective translocators isolated from tissue have been small molecular structures which appear to possess the prime function of cell membrane degradation, e.g., antibiotics, bacterial toxins, and the immune system's complement. A function which they seem to hold in common is the facilitation of membrane transfer of substances (e.g., ions) which do not normally cross the lipid barrier (Mueller and Rudin, 1969a).

Proteinoid excitation is the first indication of excitation occurring in macromolecular structures totally independent of biological natural selection. Even the peptide used by Kennedy et al. (1977) was modeled after life structures.
CONCLUSIONS

These findings indicate that the protein-lipid stereodynamics allowing for electrical activity of a very selective nature may not be processes attained only by biological natural selection, but rather functional molecular configurations which form due to the non-random ion–proteinoid interaction in non-biological milieu.

The high incidence of excitability with proteinoids indicates a possible selective advantage of those molecules over the molecular arrangements which lack such dynamic two-stable-state characteristics.

The comparable properties of excitable tissue and protocells, even to selection of similar ionic conductances and inhibition by calcium, points to possible functional commonality. The effect of calcium and the improbability of precise protocell membrane architecture forces an increased consideration of excitation as being a colloidal function with a "carrier" as opposed to "gating" mechanism.

There should be a careful assessment of the energetic relationships since the molecular evolution of these particular species implies the manifestation of significant functional "ordering" within a high entropy albeit high energy system. The implication is that "excitation" may itself be an energy transfer system allowing structural maintenance by the utilization of surrounding entropic processes. This link between excitation and energetics may be what Mueller and Rudin (1969a) are observing when they note common properties between "gated translocators" and active ion transport mechanisms.

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