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Oscillations and Spatial Nonuniformities in Membranes

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Raima Larter was born in Texas, raised in Idaho, and received a B.S. in chemistry from Montana State University in 1976. Her Ph.D. in physical chemistry was earned at Indiana University, Bloomington, in 1980. After a posdoctoral year (1980-1981) at Princeton University, she joined the faculty at Indiana University-Purdue University at Indianapolis, where she is currently Associate Professor of Chemistry. Her research interests include experimental and theoretical studies of chemical oscillations and nonlinear phenomena in membranes.

I. Introduction

In recent years we have seen a tremendous increase in the number of experimental and theoretical studies of oscillations and spatial pattern formation in membranes. One reason for the increased interest in these systems is a recently acquired appreciation for their importance to biology; another is the rapid development of experimental, mathematical, and computational tools that have opened up new opportunities for studying these systems. Sustained oscillations in neurons and pacemaker cells in the heart and secretory glands are well-known, but are also known to occur in other types of cells as well as nonliving membrane systems. In addition to oscillations, spatial patterning is often observed in these systems, sometimes accompanying growth and developmental processes. The mechanisms by which oscillation and pattern formation arise are probably related, a possibility that will be explored in detail in this article.

Outside of the enormously important implications for biological systems, oscillations and pattern formation in membranes have attracted the attention of chemists because of the interesting coupling that may occur between chemical reactions, mass transport, and other physical phenomena. Since Teorell's pioneering (1958) study[1] of electric potential oscillations in ion-exchange membranes, many related systems have been found to also display sustained oscillations, generally observed as an oscillatory membrane potential or current. Systems in which oscillations have been observed include membranes made of gel, protein, Millipore filters, phospholipid bilayers, doped polyethylene, and liquid membranes. Oscillations occur only when the system is far away from thermodynamic equilibrium achieved by imposing some sort of external force on the membrane; driving forces studied have included concentration gradients, electric fields, imposed light, pressure gradients, and others.

The experimental study of pattern formation in membranes is not as extensive as the study of oscillations. In this area, theoretical investigations have gone far beyond experimental verification of the predictions of theory. However, a number of experimental and theoretical studies have addressed the question of whether mass transport processes are different in spatially patterned, i.e., nonuniform, membranes. Since mass transport often plays a critical role in the generation of both oscillations and patterns, we will review this body of work as well and indicate possible future research directions that could tie these areas together.

This review is divided into sections covering the phenomena of oscillations (section II) and spatial pat-

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terns or nonuniformities (section III). Within each section we first briefly review the state of knowledge prior to 1979, referring the reader to earlier reviews, if any, and then discuss more recent developments in each subfield in the past decade (1979–1989). The focus of this review is on artificial membrane systems, but we will include some discussion of well-characterized biological membrane oscillations. Hence, the sections are also divided into separate subsections for biological and artificial systems; biological systems are discussed first to provide motivation for the descriptions of the artificial systems which are often designed as models of the less well-understood biological examples. We also include separate sections on the existence of chaotic behavior in these systems, on possible practical applications and uses for oscillations and spatial nonuniformities in membranes, and on implications of these studies for the broader understanding of certain important biological processes.

The most recent collection of papers on membrane oscillations and pattern formation is already over a decade old[2] (1975) and very few other relevant collections or reviews exist. The Faraday Society published the proceedings of a 1974 symposium on oscillatory phenomena[3] with one section on membrane oscillations. A more recent (1987) book[4] on chaos in biological systems contains a section of contributed papers on neuronal oscillations. As far as we know, no review of oscillations and pattern formation in biological *and* artificial membrane systems has ever appeared. Berridge and Rapp reviewed the known *biological* oscillations, including examples of membrane oscillations,[5] in 1979. A closely related area of investigation is that of oscillatory chemical reactions in bulk phases; several recent reviews have appeared[6] and the reader is referred to these for a more complete list of references.

II. Oscillations in Membranes

A. Historical Review (Prior to 1979)

1. Biological Membranes

Many examples of biochemical and cellular oscillations are known and have been extensively studied. At the cellular level, two types of oscillations exist: one type is primarily localized to events occurring in the cell membrane, while the other type occurs in the intracellular region, i.e., the cytoplasm. These two types are not mutually exclusive, however, since cytoplasmic-based oscillations, such as those associated with energy transduction, may influence a membrane-based oscillation.

Biological oscillations at the cellular level were reviewed in 1979 by Berridge and Rapp, who emphasized the role of calcium ion in coupling the membrane- and cytoplasm-localized oscillators. This species is of particular importance because of the existence of calcium-dependent ion channels in the cell membrane and the ability of calcium to induce the release of sequestered calcium in the cell interior; interaction of these two processes can lead to extensive coupling of cytoplasmic and membrane events. Other coupling avenues exist as well; for example, membrane oscillations that are driven by oscillatory ion pump activity are closely linked to metabolic activity in the cytoplasm, since the pumps require energy produced through the metabolic cycle. Since oscillations have been observed in all phases of the metabolic pathway, it is difficult to separate the cause and effect of oscillations in these kinds of systems and, hence, to categorize cellular oscillations as either "membrane oscillations" or "cytoplasmic oscillations". Nevertheless, the 1979 review attempts to distinguish examples of these two groups.

In a companion article,[7] Rapp tabulated an "Atlas of cellular oscillators" which included experimental reports of known biological membrane potential oscillations. The types of true membrane-localized oscillations known at that time included oscillations in membrane potential of secretory cells (such as pancreatic β cells), isolated neurons (particularly the molluscan variety), and certain cardiac cells (the sino-atrial node and the Purkinje fibers). Other known biological oscillations included oscillatory ion movements in mitochondria, oscillations in photosynthesis, glycolytic oscillations, and oscillations in protein synthesis. Berridge and Rapp reviewed the state of knowledge of cellular oscillations in 1979 quite thoroughly, and the reader is referred to their articles[5,7] for further details.

Here, we will focus on two historical examples of biological oscillatory phenomena that were not included in the 1979 review. Both examples are concerned with growth and development. The first example involves oscillations in potential observed near a sprouting bean root; the second does not involve oscillations per se but does involve evidence of excitability. The bean root potential oscillations were discovered over 30 years ago but have somehow escaped widespread attention. The oscillatory sprouting bean root may prove to be a relatively simple example of a large class of developmental systems that are found to display spatial nonuniformity associated with the oscillations. This aspect will be discussed in detail in section III.

In 1957, Scott discovered[8] that the electrical potential measured just outside the surface of a bean root sprouting in water oscillated with a period of about 5 min. Scott and his student I. S. Jenkinson continued to study[9–13] these oscillations throughout the 1960s and determined that a feedback mechanism was probably responsible for them. This example is particularly interesting in light of the observation reported earlier[14] (1955) by this group that the electric current generated by the growing root was directly correlated with the rate of elongation of the root, i.e., with its growth rate. Other examples of direct correlation between growth or development and bioelectric oscillations have been discovered since that time; examples include the action potentials observed during regeneration in *Acetabularia* and current pulses that accompany developing eggs of *Pelvetia*, both species of algae (see section III.B.1). The bean root example is interesting because the observed oscillations are apparently related to the spatial patterns in the potential outside the sprout, indicating that this system may be an example of a spatiotemporal chemical instability.

A second group of phenomena studied prior to 1979 but not covered in the Berridge and Rapp review is, in hindsight, intimately related to the phenomena of membrane oscillations and excitability. Egg cells of most, or all, types have a cellular membrane that is excitable in the sense of being capable of generating single action potentials. These cells possess current–voltage relations containing a region of negative conductance[15] and display the property of bistability of membrane potential, both phenomena associated with excitable cells such as neurons or pacemakers. These properties seem to be used by the egg in the generation of a very fast change in membrane potential upon fertilization by a sperm cell; this provides, at least in some systems, an electrical block to the penetration of the egg by more than one sperm, a phenomenon known as polyspermy. The excitability properties of the egg cell membrane are, thus, very important in ensuring proper development of the organism.[16,17]

An example is the membrane of the egg cell of the starfish *Mediaster aequalis*, which shows an action potential when deliberately depolarized. Hagiwara et al. measured the current–voltage curves[18] for this system in 1976 and found a region of negative conductance under the conditions of the depolarization experiment. The egg cell excitability appears to be due to the same type of electrical phenomenon as that observed in spontaneously oscillatory excitable cells, such as cardiac pacemaker cells and neurons, which also possess a current–voltage curve with a region of negative conductance. The theoretical interpretation of this feature is discussed in section II.B.3.

Although the egg cell membrane is not normally thought of as sustaining oscillations during the normal course of events, it may be that the membrane of an egg cell *is* spontaneously oscillatory and that these oscillations are of fundamental importance. In 1978, Yoneda et al. discovered periodic vibrations in the surface tension of the membranes of unfertilized, but artificially activated, sea urchin eggs[19a] from which the cell nucleus had been removed. (A more recent experiment by Cuthbertson and Cobbold[19b] revealed [Ca^{2+}] oscillations following sperm fertilization of superovulated mouse eggs; these may be related to a membrane oscillation.) The period of oscillation in the Yoneda experiment was approximately equal to the period of the cell division cycle that normally occurs in fertilized eggs. From this observation, the investigators speculated that the development of the egg following fertilization, which proceeds via periodic cell division, may be mediated by a periodically varying cell membrane surface tension and that the periodicity is *not* due to an inherent property of the chromosomal apparatus in the cell nucleus. This example may provide a link between membrane oscillations and the rhythms that occur at a higher organizational level in biological systems such as the cell division cycle, organismic-level biorhythms, and other circadian rhythms. This link is still highly speculative, however.

2. Artificial Membranes

We now turn to a review of the artificial membrane oscillations known as of 1978. The study of these systems was primarily motivated by a desire to understand the basic physicochemical phenomena that occur during biological membrane oscillations. The artificial systems are, of course, much simpler than the biological systems, but the underlying assumption in all of this work is that the artificial system retains the *important* features of the biological system and only eliminates the *unimportant* details. Nevertheless, the features that are deemed to be important in biology are almost as numerous as the investigators who have tackled the problem. In addition, as in most biomimetic research efforts, the artificial membrane systems have become interesting objects for study in their own right and have provided important information about the general phenomena of physicochemical instabilities such as oscillations, chaos, and spatial pattern formation that occur in systems other than just membranes.

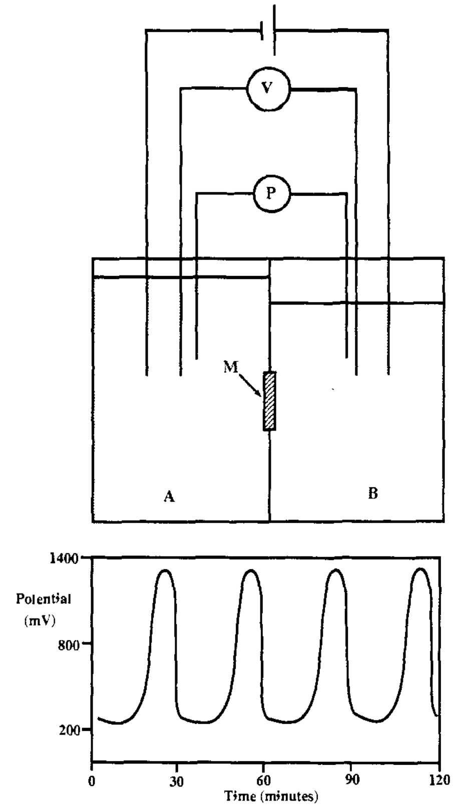

Figure 1. (Top) Schematic drawing of membrane cell used for studies of oscillations. Solutions A and B generally contain electrolyte, sometimes at differing concentrations. The membrane M may be porous fritted glass or an ion-exchange gel membrane (in the Teorell oscillators[1]) or may be composed of a lipid bilayer, a Millipore filter doped with a lipid analogue, polyethylene doped with iodine, a gel with immobilized enzyme, and others. The osmotic pressure is monitored or controlled with P, a pressure transducer, the membrane potential is monitored with V, a voltmeter, and a current may be applied with a constant-current source as shown. (Bottom) Qualitative behavior of oscillations observed in the Teorell oscillator; curve shows order of magnitude of amplitude and period but is not quantitative. (Adapted from ref 23.)

(*a*) *Teorell Oscillators.* The first systematic study of sustained potential oscillations in an artificial membrane system was carried out by Torsten Teorell and reported[1] in 1958. The systems Teorell studied[20–23] from 1958 to 1962 consisted of a porous glass or ion-exchange membrane separating two electrolyte solutions of differing composition. Upon driving a constant electric current through the membrane, oscillations in the membrane potential, resistance, and hydrostatic pressure were observed (see Figure 1). Teorell's work generated a great deal of discussion in the electrophysiology community at the time[24] and was widely discussed as a possible physicochemical model for the action potential in neurons.

In the systems studied by Teorell, the steady-state current–voltage (*I–V*) relation at fixed pressure is found to contain a negative conductance region (see Figure 2).

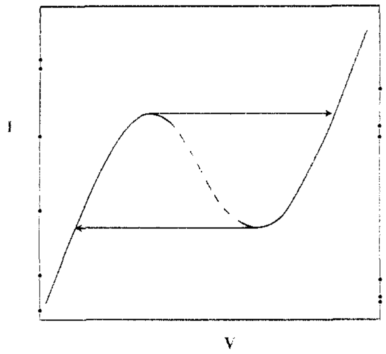

Figure 2. Current-voltage (I-V) curve for membrane oscillator. Arrows show discontinuous jumps in voltage that occur when current is varied beyond the extrema in the curve. Dashed portion is the negative-conductance region which corresponds to an unstable state of the membrane. Oscillations occur when the current is driven around the hysteresis loop shown. (Adapted from ref 22.)

An application of Ohm's law, $I = GV$, would assign a negative slope G to the dashed portion of the curve, although, of course, Ohm's law, a linear equation, does *not* describe the nonlinear I-V curve shown in Figure 2. The nonlinearity is, in fact, a critical feature of the mechanism by which oscillations arise. The hysteresis loop shown would describe a process by which the current increases slowly over a certain range of voltages and then rapidly jumps to the high-voltage region, whereupon the current starts a slow descent before hopping suddenly back to the low-voltage region. The possible mechanisms by which the current and voltage could be driven around this hysteresis loop will be considered in the following sections.

In 1961, Chaim Forgacs, apparently unaware of Teorell's discovery, also observed[25] oscillations in membrane potential upon passing a current through a cation-exchange membrane separating identical solutions of silver nitrate. Forgacs also experimented with a dialysis cell containing agar gel rather than aqueous electrolyte solution, to minimize convective effects, and found oscillations of even larger amplitude.

(*b*) *Doped Polyethylene.* Following Teorell's pioneering studies, other investigators observed similar phenomena in different types of membranes. In 1967, Swan observed[26] that when an electric field larger than 4×10^7 V/m was applied to a polyethylene film doped with I_2, the current through the film developed oscillations. It was previously known[27] that the electrical conductivity of thin polyethylene film increases by several orders of magnitude when iodine is absorbed from aqueous sodium iodide. It appears that electron transfer from the polyethylene molecules to vacant acceptors in the iodine molecules generates mobile "holes" in the polymer matrix, enhancing conduction in what would otherwise be an insulator.

The mechanism by which oscillations arise in this system was proposed by Jones and Lewis[28] to consist of the establishment by the charge-transfer process of space-charge domains that propagate all at once across the film. Jones and Lewis also found that a similar system produced by infusing dry polyethylene with iodine vapor exhibited an I-V curve with a negative slope region and also produced oscillations. Similar oscillations were observed by Swaroop and Predecki[29,30] and by Toureille et al.[31] in dry poly(ethylene terephthalate), polystyrene, and also polyethylene.

TABLE I. Excitable Gel Membranes

monomer	polymerization method	ref
glycerol monooleate and 5-10% oleic acid brushed over cellophane	exposure to air	36
linseed oil (mixture of glycerol linoleate and glycerol linolenate)	droplet spread on oxidative $KMnO_4$ solution	39
glycerol monooleate and succinic (or phthalic) anhydride	under vacuum for 24 h at 100 °C	45
1-2% bitumen + 1% oleic acid in benzene	droplet spread on water	45
collagen sheet	treat with lauryl sulfate	46

(*c*) *Lipid Membranes.* The early and continuing interest in artificial models of biological membranes led to a number of investigations of oscillations in membranes composed of lipid bilayers. These were attractive for study since they were simpler than biological membranes which are also composed of a lipid bilayer, albeit a much more complex one containing proteins, etc. Mueller and Rudin found that the lipid bilayers they prepared were not electrically excitable unless an "excitability inducing material", or EIM, was added to the bilayer.[32-34] This mysterious material, extracted from egg whites, is, in retrospect, probably some sort of channel-forming protein, although its exact identity is still unclear to this day. The important aspect of Mueller and Rudin's discovery was that excitability could be definitely tied to the membrane alone, and elaborate cellular structures did not need to be invoked. This important discovery gave incentive to other investigations of artificial lipid systems to determine the possible sources of excitability.

Pant and Rosenberg reported[35] in 1971 evidence for electrical oscillations across a lipid bilayer membrane containing no EIM or other channel formers and separating KCl solutions that contained a redox couple. The KCl solution on one side of the membrane also contained KI at pH 10, while the other solution contained potassium ferricyanide at pH 5. Oscillations in the membrane potential were observed when a current was driven through the lipid bilayer. Oscillatory physical movements of the bilayer were also observed and occurred in synchrony with the electrical oscillations.

The main problem with studying oscillations in lipid bilayers is that the membranes are often fragile and difficult to work woth. Throughout the 1960s and 1970s, Monnier and co-workers in France developed stronger lipid-like membranes by polymerization of oils.[36-45] One example they studied is constructed[45] by spreading a drop of an unsaturated fat, such as linseed oil, upon an oxidizing solution of $KMnO_4$. The thin layer undergoes oxidative polymerization and becomes sufficiently strong so that it can be removed from the liquid surface and placed between two salt solutions. The resulting membrane shows some excitability properties including action potentials and regular oscillations in voltage when a constant current is applied. Table I shows the different types of membranes studied by this group and others[46] which display excitability.

The technique of polymerizing oils to produce a stronger membrane has not become a widespread one for the study of oscillations, although the technique is still used to produce a strong lipid-bilayer-like membrane for other purposes. An alternative technique of doping a porous solid membrane with lipids or lipid analogues also produces a strong membrane with many of the same properties as a single lipid bilayer, and oscillations in these systems have been studied quite extensively. In 1968, Shashoua reported[47] erratic, spiky jumps in membrane potential and unusual current–voltage curves in a bipolar membrane made by layering a polycation phase onto a polyanion phase. Later studies[48] showed that these oscillations could be made to be periodic and regular. The interface region is analogous to a p–n junction and the mechanism of oscillation is thought to be similar to that of a semiconductor. Further refinement of the technique for preparing the bipolar membrane involved synthesizing a porous polymer membrane with pores ranging from 50 to 3000 Å in diameter.[49] The cationic and anionic phases are then placed into the pores in layers. Various species were used for the cationic and anionic phases. The cationic species included poly(lysine) HBr, poly[(dimethylamino)ethyl acrylate], Ca^{2+}, Ba^{2+}, cytochrome *c*, poly(L-sarcosine), and poly(L-lysine) HBr. Anionic species utilized were poly(glutamic acid), poly(acrylic acid), yeast RNA, dextran sulfate, acrylic acid/acrylamide copolymer, acrylic acid/methyl acrylate copolymer, poly(glutamic acid), and DNA. Oscillations in potential are observed when these membranes are placed between identical 0.15 M NaCl solutions and a current is passed through them.

Studies carried out in Japan at about this time (1973) involved considerably simpler systems in which the pores of the filter are filled with a single molecular species, usually dioleyl phosphate, $[CH_3(CH_2)_7CH{=}CH(CH_2)_7CH_2O]_2POOH$, or DOPH. Since regular oscillations are observed in this single-component system as well, the existence of a p–n type junction is apparently not a critical feature. Oscillations in membrane potential were first observed[50] when the lipid analogue doped filter was placed between solutions of differing concentrations and a pressure gradient of about 20 mmHg was imposed. The mechanism of oscillation in these systems was (and is) thought to involve the formation and destruction of different lipid phases in the pores of the filter, a mechanism that had been predicted[51] in 1972 on the basis of an observed hysteresis of membrane permeability with concentration.[52,53] This system and similar lipid analogue doped filters such as one constructed from glycerol α-monooleate in a nitrocellulose film[54] have provided many examples of lipid membrane oscillators and continue to be studied, mostly in Japan. A particularly interesting early study[55] showed that varying the amount of lipid contained in the pores produced changes in the oscillation pattern that involved the introduction of a second frequency, a phenomenon now known as "bursting". More recent investigations of these systems are described in section II.B.

(*d*) *Immobilized Enzymes.* A study by Naparstek, Thomas, and Caplan[56] in 1973 of papain enzyme immobilized in a collodion membrane coated on a glass electrode revealed spontaneous pH oscillations at the membrane/glass interface. Subsequent investigation showed that membrane potential and current oscillations were associated with the pH oscillations. Determining the origin of the oscillations in an enzyme system is somewhat more straightforward since the feedback mechanism can be usually identified by knowing the enzyme kinetics involved. In the original experiment involving a membrane coated on the tip of a glass electrode, the positive feedback due to autocatalysis of the enzyme reaction is combined with a negative feedback due to diffusion of the substrate through the film. As will be discussed in section II.A.3.d, the interplay of these two effects produces oscillatory behavior.

(*e*) *Liquid Membranes.* A particularly influential paper appeared[57] in 1978 in which Dupeyrat and Nakache reported oscillations in a liquid membrane system (a water/oil interface). One intriguing feature of Dupeyrat and Nakache's discovery was the apparent coupling that existed between the electrical properties of the membrane,[58] the chemical reaction involved, and the surface tension of the interface. A similar system was studied by Guastella.[59] Large-amplitude mechanical oscillations were also observed in the Dupeyrat–Nakache system, leading to some speculation about its relevance as a model for locomotion in unicellular organisms. The mechanism of *oscillations* is now thought to be inherently chemical, not hydrodynamic, and any influence of hydrodynamic effects is not primary. The phenomenon of *movement* of the liquid interface, however, is clearly hydrodynamic in origin and will not be considered in this review. The 1978 report[57] was influential in that it spawned a large number of studies of related systems; this body of more recent work is reviewed in section II.B.2.c.

3. Theoretical Studies

In the nonlinear dynamics area it is not uncommon to find that the theoretical analysis of problems is as extensive or even more extensive than the experimental studies. Some theoretical work was reported prior to 1978, but most of it was confined to models of oscillations in membranes containing immobilized enzymes. A few other papers appeared as well, and these are discussed in the following sections.

(*a*) *Teorell Oscillators.* Teorell proposed a mechanism for the electrical oscillations observed in his systems; in this scenario, the superposition of electrochemical and hydrostatic driving forces is critical. One assumption, which introduces the necessary nonlinearity, is an ad hoc relationship between the steady-state resistance of the membrane and the bulk flow through the membrane. Theoretical current vs resistance curves derived from this model[21] for fixed pressure show an N-shaped characteristic in which the middle branch is unstable; in such a system, hysteresis would occur and variations in pressure would drive the system through periodic cycles around the two stable branches of the current–resistance characteristic. Experimentally, hysteresis *was* observed in the current vs resistance relationship at fixed pressure, and oscillations were, of course, observed when the pressure was allowed to vary.

Aranow, in 1963, was able to suggest[60] a physical basis for Teorell's proposed mechanism. She derived all of Teorell's equations from first principles, a procedure that did not require an ad hoc assumption regarding the source of nonlinearity. The important new feature that becomes apparent with this approach is the notion that the clamping force due to fixed pressure acts like a body force in the membrane's equation of motion. The results of this study showed conclusively that the oscillations observed in the Teorell system were really hydrodynamic in origin and a consequence of the effect of the boundary conditions on the hydrodynamic stability. This, of course, does not mean that all membrane oscillations are hydrodynamic in origin but, rather, that phenomena due to hydrodynamic effects can be qualitatively similar in all *important* details to those due to chemical, or other, mechanisms.

In 1970, Kobatake proposed[61] a flip-flop mechanism, i.e., one involving relaxation kinetics, for the Teorell oscillations and showed the close relationship between the origin of these membrane potential oscillations and the equilibrium phase transition in a van der Waals gas. Kobatake also showed how to treat these oscillations in the context of the generalized entropy production ideals proposed by Glansdorff and Prigogine.[62] In Kobatake's model, transition between two steady states, corresponding to high and low values of membrane resistance, takes place when the generalized entropy productions of the two steady states reach the same value.

Meares and Page, in 1974, published[63,64] a detailed model of the Teorell oscillator that emphasized the electrochemical nature of the system; results of their calculations with this model agreed quite well with experiment and showed how features of the oscillation were related to membrane parameters such as density of fixed charge, etc. One interesting result of their derivation is a formula for the period of oscillation in terms of these characteristic membrane parameters as well as those which describe the surrounding electrolyte solutions. As discussed in section IV, this formula could be used to design a device for quantitative analysis; the proposed device would consist of a Teorell oscillator whose frequency would be related to the amount of an unknown in the bathing solution through the Meares–Page formula.

(*b*) *Doped Polyethylene.* The mechanism by which the oscillations in iodine-doped polyethylene arise is thought to involve electron transfer from the polyethylene to acceptor levels in the iodine molecules; this idea was discussed by Jones and Lewis[28] in 1974. The charge that is transferred accumulates as a space charge region until a certain threshold is reached; once this threshold is passed, the region propagates as a mobile domain, giving rise to a current surge. The charge then begins to accumulate again, the mechanism is repeated, and an oscillatory current is observed. No detailed equations of motion or quantitative theoretical studies of this system have been reported, however.

(*c*) *Lipid Membranes.* (*1*) *Bilayers.* A mechanism for the oscillations observed by Pant and Rosenberg was proposed by Karvaly[65,66] in 1973. The mechanism involved a transfer of electrons from I^- to the bilayer, followed by diffusion of unspecified charge carriers across the membrane. At the other interface, transfer of electron holes from the ferricyanide ion, $Fe(CN)_6^{3-}$, to the charge carriers in the bilayer, followed by diffusion of the electron holes back across the bilayer, completed the cycle. The feedback that leads to oscillations involves a regeneration of I^- and $Fe(CN)_6^{3-}$ by the products of the original charge-transfer reactions, I_2 and $Fe(CN)_6^{4-}$. This mechanism involves, then, two coupled feedback loops (the coupling is due to diffusion of charge carriers). In light of recent theoretical developments in dynamics, this coupled oscillator mechanism should give rise to complex, or mixed-mode, oscillations; these oscillations are, indeed, observed in the published results of Pant and Rosenberg. The membrane potential oscillations are composed of alternating large-amplitude and small-amplitude peaks; this type of complex, or mixed-mode, oscillation is also referred to as a bursting oscillation and is of great current interest because of the propensity of such periodic oscillations to become chaotic. This is discussed further in sections II.A.4 and II.B.4.

Another group of early theoretical studies was concerned with the importance of phase transitions and domain formation in lipid bilayers in the mechanism by which oscillations and other instabilities, such as action potentials, may arise in these types of membranes. Sanfeld and co-workers proposed[67] a coupling between surface chemical reactions and the membrane potential as the source of deformations of the membrane, such as occur in the Dupeyrat–Nakache liquid membrane. McQuarrie and Mulas[68] showed that charge is often distributed asymmetrically across a lipid bilayer, a feature that will also be coupled to the membrane potential, and derived a statistical theory for this phenomenon. Kobatake et al.[69] and Yoshida et al.[70] also discussed the importance of phase transitions in lipid bilayers in mechanisms by which oscillatory behavior might arise. Sackmann and co-workers[71] published an early account on this subject as well. Cotterill proposed[72] that the electric field associated with the membrane potential can have an effect on the melting point of a lipid bilayer and that this phase transition change may be important in the origin of the action potential. These ideas all build on what was previously known regarding the phases of lipid bilayers,[73] the importance of phase transitions in the effect of anesthetics that act at the cell membrane,[74] and the importance of phase transition and fluidity of the lipid bilayer in the major functions of a cell membrane.[75] These theoretical ideas were clarified and expanded in the past decade; see section II.B.3.e for further details.

(*2*) *Doped Filters.* Katchalsky and Spangler gave[76] in 1968 an extensive and influential discussion of the origin of membrane potential oscillations which focused on the mechanism of the oscillations observed by Shashoua[47–49] in a polymer filter doped with lipids of two types. Their remarks were of broader significance, however, and apply equally well to a general membrane oscillator. The importance of a negative conductance region in the current–voltage relation and the close relationship between the membrane systems and other chemical systems that display the phenomenon of metastability was emphasized.[76] They also discussed the relationship between oscillations and phase transitions, describing the origin of oscillations in terms of a hysteresis loop around an S-shaped curve. Their remarks received wide exposure and influenced many subsequent investigators of membrane oscillations.

Yoshida, Kobatake, and co-workers published a number of theoretical studies[51–53] of DOPH-doped filters, some prior to the discovery of oscillations[50] in these systems. It was experimentally known that these filters displayed hysteresis[51] in the permeability of salt, which was traced to a phase transition occurring in the DOPH bilayers and multilayers in the pores of the filter. On the basis of this observation, they predicted that this system might become oscillatory if it could be driven back and forth through the metastable region; oscillations were, in fact, subsequently found as a result of this preliminary theoretical work. The study of this system is a good example of the interplay between experiment and theory that has been so successfully applied to elucidating the origin of oscillations and other complex nonlinear behavior in chemistry as well as other fields.

(*d*) *Immobilized Enzymes.* Caplan, Naparstek, and Zabusky (1973) published results[77] of a computer simulation of a model of immobilized papain enzyme, a catalyst for the hydrolysis of benzoyl-L-arginine ethyl ester. Their results showed that the observed oscillations were due to the coupling of positive and negative feedback loops in the system; the positive feedback is due to the autocatalytic enzyme reaction while the negative feedback results from the diffusion of the substrate through the membrane to the enzyme site. A similar study was carried out by Zabusky and Hardin[78] (1973); a boundary layer for one of the diffusing species was included in this study and was found to affect the period of oscillation.

Hahn, Ortoleva, and Ross[79] (1973) emphasized the importance of variable permeability as a source of feedback in a study of several models for membrane transport. The focus of their investigation was primarily on the control of permeability of one species by another (such as the Ca^{2+}-dependent K^+ pump in nerve cell membranes) although self-control of membrane permeability by a transported species was also considered. The model predicted the existence of multiple steady states, hysteresis, and oscillations. Their general result was that the complexity of the enzyme-catalyzed reaction mechanism necessary for the production of nonlinear behavior is reduced if the enzyme is immobilized in the membrane and variable permeation plays a role.

Blumenthal[80] (1975) published a very complete study of a model of an immobilized oligomeric enzyme. The enzyme consisted of n subunits and mediated transport by binding permeants asymmetrically; i.e., the binding of permeants was stronger on one side of the membrane than the other. Oscillatory behavior was found and, in addition, the possibility of spatial patterning was shown to exist in such a system if lateral diffusion were included. [The spatial patterning will be considered further in section III.] Osawa and Tada[81] (1977) studied the conditions for oscillation in Blumenthal's model for the case in which the oligomers functioned in an extremely asymmetric fashion, i.e., for which the binding was infinitely faster on one side than on the other. With this modification, they were able to investigate the qualitative conditions for limit cycle oscillations and action potential overshoots. The latter behavior emerges only in the case considered, i.e., when the membrane is extremely asymmetric. Tagami[82] (1978) modified Blumenthal's model by including a second molecule beyond the transported substrate which mediated the binding of substrate to the oligomeric units. The inclusion of this second species as a dynamical variable causes the flux of substrate through the membrane to be a nonlinear, rather than a linear, function and ensures that the model exhibits saturation. The model also exhibits selectivity of the transported species.

(*e*) *Liquid Membranes.* Dupeyrat and Nakache[57,58] proposed a mechanism for the oscillatory liquid membrane potential and interfacial waves observed in the membrane oscillator they discovered. The proposed mechanism involved the formation of an ion-pair complex between the large surfactant cation and the picrate anion at the position of the liquid–liquid interface. A relevant theoretical paper[83] showed how a deformational instability could arise from coupling of mass transfer chemical reactions with the interfacial tension. A more detailed understanding of the specific mechanism of the Dupeyrat–Nakache system was not achieved for several more years; these advances will be described in section II.B.3.b.

4. Chaos

In recent years, chaotic behavior has been discovered to occur in a wide variety of systems. The meteorologist Edward Lorenz[84] was the first to propose, in 1963, that deterministic systems could display random, chaotic behavior but that order existed within this chaos in the form of a strange attractor. Lorenz studied this phenomenon in hydrodynamic systems. Since his early work, chaos has been found in settings ranging from ecology[4] and the motion of the solar system[85] to inorganic redox reactions[86] and neurons.[4] It is the latter type of system that is, of course, of interest to us here.

The mechanisms by which this chaotic behavior arise are now beginning to be elucidated. In many of these systems, chaotic behavior is just one of several behavioral modes available; other modes may include steady-state behavior and periodic oscillatory behaviors of different kinds. In particular, the existence of more than one fundamental frequency of oscillation is thought to be a necessary condition for the existence of chaos. In a common scenario, chaotic behavior arises when two or more oscillators in the system are coupled in a nonlinear way. Often, oscillatory behavior can be discerned in which the multiple frequencies are identifiable. This type of oscillatory behavior is characterized by mixed-amplitude oscillations, i.e., oscillations in which the system variables go through some number of large-amplitude oscillations followed by some number of small-amplitude oscillations. The periods of time separating peaks of different amplitudes determine the multiple frequencies in the system. The most recent studies of chaotic behavior in membrane systems will be reviewed in the following section. Here we focus on a few early papers that show evidence for the possible existence of chaos in both artificial and biological membrane systems. This evidence often consists of the identification of multiple frequencies via mixed-amplitude oscillations in the reported behavior but chaos, per se, was usually not explicitly considered, especially in the earlier reports.

The oscillations observed across iodine-doped polyethylene are of the quasiperiodic type when a high dc electric field (>1 kV/mil) is applied[26] (Swan, 1967). A quasiperiodic response is one in which the two frequencies are related by an irrational number. In addition, a possible phase-locked state (one in which the

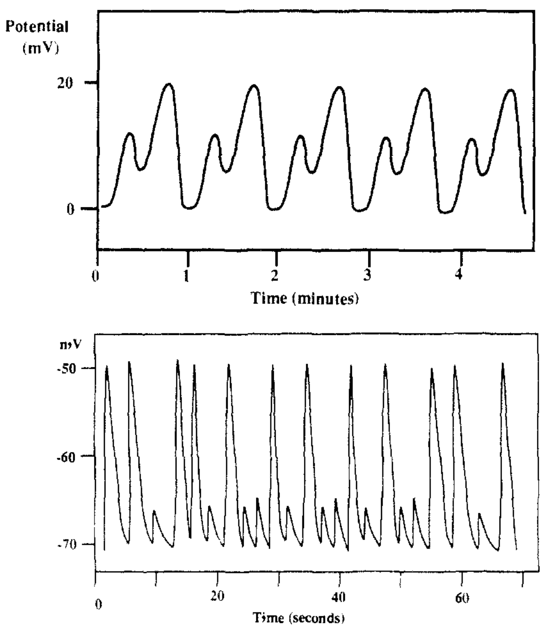

Figure 3. (Top) Qualitative behavior of potential oscillations in Pant–Rosenberg oscillator (adapted from ref 35). Curve shows order of magnitude of amplitude and period but is not quantitative; existence of second frequency is indicated by small- and large-amplitude peaks within each cycle. (Bottom) Qualitative behavior of possible chaotic oscillations observed in DOPH-doped Millipore filter membrane. Bursting nature of oscillations is shown, as is order of magnitude of amplitude and period. (Adapted from ref 55.)

ratio of the two frequencies is a rational number) is also reported in this early work, also occurring at high field strengths. These two behaviors are strong evidence that chaos could also exist in this system, although the early date of the study precludes any mention of this possibility.

Another early paper (Pant and Rosenberg,[35] 1971) shows evidence for the existence of multiple frequencies in the lipid bilayer redox couple system that was studied by these investigators. In this case, the oscillations clearly show the existence of multiple amplitudes and frequencies (see Figure 3 (top)). No chaos is reported in these studies because, again, the science of chaos was not well established at that time.

Another early study that, in retrospect, indicates the possibility of chaotic behavior was carried out by Arisawa and Furukawa[55] (1977). This study was one of the first involving the important DOPH-doped Millipore (8 μm) filter paper described in section II.3.c.2. In this study, the membrane was placed between KCl solutions of differing concentrations and a pressure gradient was applied. Simple oscillatory behavior involving a single frequency was observed when the amount of DOPH present in the pores of the filter was low. The amount of DOPH (Q) was varied systematically and it was found that for $Q > 6.5$ mg/cm^2 the oscillation pattern developed a second frequency. This appeared as small-amplitude spikes interspersed among the larger amplitude spikes (see Figure 3 (bottom)), which were observed at low Q. This paper[55] is important because it identifies Q as a controllable bifurcation parameter; variations in this parameter can be used to take the system from a simple oscillatory state to a complex one and, possibly, onto chaotic behavior. Again, at this early date, the possibility of chaotic behavior was not investigated systematically.

Early studies of biological systems that are relevant to the later studies of chaos often involved application of external oscillatory perturbations to inherently oscillatory systems, such as pacemaker neurons and the like. Some of the systems studied include flutter vibrations applied to mechanoreceptors in the hand[87] (Talbot et al., 1968), low-frequency tones imposed on auditory fibers[88] (Rose et al., 1967), sinusoidal forcing of a mechanoreceptor[89] (French et al., 1972), synchronization and phase-locking via perturbations of the *Limulus* lateral eye[90,91] (Ascoli et al., 1974, 1977), and the application of ion-transport blockers such as strychnine[92] and Fluarepam[93] to neurons (Hoyer and Klee et al., 1978). In addition to these studies involving perturbation of spontaneously oscillatory systems, Yoneda et al.[19] (1978) observed that unfertilized sea urchin eggs display periodic changes in surface tension; Figure 3E in their paper indicates the possibility of multiple frequencies in these oscillations. The method of perturbation by sinusoidal forcing and application of ion-transport blockers continues to be an important means of studying chaos in biological systems[4] as will be discussed further in the following section.

B. Recent Studies (1979–1989)

1. Biological Membranes

Researchers have continued to study the cellular oscillators reviewed by Berridge and Rapp in 1979 and have discovered many new examples. Since the focus of this review is primarily on artificial membrane systems which are studied as models of these biological membrane oscillators, a complete review of all new biological membrane oscillators discovered in recent years will not be attempted here. However, a few representative and illustrative examples will be mentioned.

Investigators have continued to study self-sustained oscillations in neurons, algae, and excitable cells from sources such as cardiac tissue and muscle. For example, peridic oscillations in the membrane potential of cardiac cells[94] (Matsuda et al., 1982) and mouse fibroblasts[95] (Oiki and Okada, 1988) have recently been reported. The large internodal cell of *Chara*, a species of algae, produces oscillations when stimulated by light[96] (Ogata, 1987) and even a protoplasmic droplet taken from this species can be induced to oscillate when treated with appropriate chemicals[97] (Toko et al., 1985). The latter phenomenon is thought to be due to the reconstruction of a lipid membrane from the protoplasm, which is subsequently caught up in a peridic formation–destruction cycle. By far the most widely studied biological membrane oscillations are those which occur in neurons. The squid giant axon produces regular oscillations when bathed in solutions with ionic composition that differs from the natural extracellular fluid in the squid and these oscillations have been extensively studied (see, for example, Aihara and Matsumoto[98,99]). Neurons isolated from other molluscs have also provided subjects for the study of similar phenomena.[100-103]

An interesting example of the type of information that can be gleaned from studies of neuronal oscillations is that of the lamprey eel neuron. The whole brainstem–spinal cord system of the lamprey eel can be maintained in vitro. By adding *N*-methyl-D-aspartate (NMDA) to the bathing solution, fictive locomotion can be achieved; i.e., a firing pattern in the neural network can be observed that is identical with that observed during true locomotion (swimming). During this fictive locomotion, some of the neurons display membrane potential oscillations that are in-phase with the motor activity. Grillner and co-workers have determined[104-106] that these oscillations continue even after the synaptic interactions are blocked (by tetrodotoxin, TTX), indicating that the oscillations arise from single neurons. NMDA-receptor activation induces a region of negative slope conductance in the current–voltage relationship in the lamprey, an effect that has also been observed in neurons of mammals; as described in section II.B.3.c, a negative slope conductance is also found in cells with pacemaker-like membrane potential oscillations, in cells with bistable membrane potential, and in artificial membrane oscillators and is a very important aspect of excitable systems.

Many of the more recent studies of periodic activity in excitable cells have focused on bifurcations, or transitions, to complex oscillations and chaos that can be made to occur by the application of a sinusoidal stimulating current to an excitable cell in a self-sustained state of regular, periodic, oscillatory activity. Many types of systems, including the eye[90,91] (Ascoli et al., 1974, 1977), auditory fibers[88] (Rose et al., 1967), and mechanoreceptors[87,89] (Talbot et al., 1968; French et al., 1972) from the monkey, show phase-locking responses; i.e., the cellular oscillation is entrained by the forcing oscillation. More recently, studies of neurons (from molluscs[100-103] and squid[98,99]) and of excitable algae[107,108] have revealed that this forcing can lead to complex behavior, including period doubling, quasiperiodicity, intermittency, and chaos. These studies are reviewed in section II.B.4.

2. *Artificial Membranes*

(*a*) *Lipid Membranes.* (*1*) *Bilayers.* Perhaps the most relevant of membrane materials as models of biological membranes, artificial lipid bilayers have been extensively studied for a number of different reasons. A large community of investigators studies the phase transition behavior of pure lipid and mixed lipid bilayers, the permeability of these bilayers, the relationship of permeability to macroscopic state of the bilayer, the behavior of proteins and channel formers that have been reconstituted in lipid bilayers or vesicles, and a whole host of other phenomena involving the use of bilayers as models for cellular membranes.

In addition to the intense general interest in lipid bilayers, a number of experiments have been reported indicating a continuing interest in oscillatory behavior in these systems. The work of Pant and Rosenberg[35] (see section II.A.2.c) was the first to show that lipid bilayers *without added channel-forming proteins* could display oscillatory behavior. As discussed above, spontaneous action potentials and rhythmic oscillations had been observed prior to the Pant–Rosenberg study by Mueller and Rudin[32-34] in an artificial lipid membrane into which EIM, a channel-forming protein, had been introduced.

Antonov et al. reported[109] in 1980 an important experiment that showed the relationship between transmembrane current and voltage fluctuations of the type normally associated with channel-forming substances, action potentials, and the like, and the phase transition behavior of the lipid bilayer. Unmodified distearoylphosphorylcholine planar lipid bilayers suspended in holes in a Teflon wall between two identical buffered salt solutions produced square-wave current fluctuations when the membrane was held at the phase transition temperature; these fluctuations were *not* observed above or below this temperature. The implication of this experiment is that ion channels can be formed by cooperative lipid domain interactions which occur when the membrane is at the phase transition temperature. This result may have significant consequences for other temperatures as well if additional species (proteins, alcohols, etc.) are present that perturb the local state of a lipid bilayer, causing "local phase transitions" or domain formation to occur. Voltage and current fluctuations in the normal cell membrane may be the result of *current transport through the lipid bilayer itself* rather than through or near the ion channel. These current fluctuations might become amplified, resulting in action potentials, rhythmic pacemaker-type oscillations, etc. A scenario similar to this has been proposed as the mode of action of the natural nerve axonal membrane by Lahiri et al.[110,111] and Cotterill.[72,112] The experiments of Antonov et al.[109] support this conjecture.

Recently, Yoshikawa et al.[113] (1988) reported observations of fluctuations and pulsating current across a planar lipid bilayer composed of L-α-dioleoylphosphatidylcholine suspended in a 1-mm hole in a Teflon wall between 0.5 M KCl solutions. The magnitude of the fluctuations was of the order of 6 pA when a voltage of 50 mV was applied, which would correspond to a fluctuation in conductivity of about 120 pS. Fluctuations of a like magnitude were observed for the same bilayer clamped by a capillary pipet in the popular "patch-clamp" technique used for the study of single channels. Yoshikawa et al. note[113] that the order of magnitude of the fluctuations observed in their system is significant, since fluctuations of only 25 pS reported by other groups for sodium channels incorporated into a planar bilayer were attributed to gating due to the channel-forming proteins. These findings along with more recent ones of Antonov et al.[114] indicate that a closer scrutiny of the role of the lipid bilayer and its phase transition behavior is needed in interpreting the data on studies of ion channels in biological membranes.

(*2*) *Doped Filters.* The technique of doping porous filters with lipids or lipid analogues has greatly increased the diversity of membranes that can be studied by improving their durability and ease of fabrication. A popular lipid analogue used in many of the early experiments was dioleyl phosphate, or DOPH, usually absorbed into the pores of an 8-μm Millipore filter. As shown in Table II, many experimental studies[50,54,116-125] of this system continue to be reported, and it appears that oscillations can be easily achieved by varying operating condition parameters such as the concentration gradient across the membrane, the applied current density, and the imposed pressure gradient. The DOPH system is attractive for study since an unambiguous link between the observed oscillations and a phase transition of the lipid within the pore has been well established.

A similar mechanism for the origin of oscillations in a system with a different lipid analogue, sorbitan monooleate, or Span-80, has also recently been demonstrated.[125] Two other lipid analogues[121-123] and one

TABLE II

DOPH-Doped Filters

bathing solns	external force	ref
7.8 mM KCl and 6.5 mM KCl	sudden concn change	50
15.6 mM KCl, both sides	2.1 V	50
20 mM KCl, both sides	50 mV	54
5 mM KCl and KCl, 5–100 mM	pressure of 20 mmHg	55
5 mM KCl and 100 mM KCl	nothing *or* current of 0.2–0.3 μA	116
5 mM KCl and 100 mM KCl	nothing *or* pressure of 33 cm H_2O and current of 0.6 μA	117
5 mM KCl + additive[a] and 100 mM KCl	pressure of 35 cm H_2O and current of 0.3 μA	118
100 mM NaCl and 100 mM KCl	nothing	119
100 mM NaCl, both sides *or* 100 mM KCl both sides	current of 0.01–0.35 μA	119
identical KCl solns	current of <0.1 μA and pressure of 10–35 mmHg	120
5 mM KCl + additive[b] and 100 mM KCl	pressure of 10–35 mmHg	120

Filters Doped with Other Lipid Analogues

lipid analogue	filter	bathing solns	ref
glycerol α-monooleate (monoolein)	large-pore filter paper[c]	0.5 M NaCl and 0.5 M KCl	121
triolein	large-pore filter paper[c]	0.5 M NaCl and 0.5 M KCl	122, 123
C_nN^+-PSS^- [d]	0.025-μm Millipore filter	1 mM KCl and 100 mM KCl	124
sorbitan monooleate (Span-80)	large-[c] or small-pore[e] filter paper	0.5 M KCl and 0.5 M NaCl	125

[a] Additives were extra KCl, $CaCl_2$, sucrose, and HCl (individually). [b] Additives were divalent cations, Ca^{2+}, Ba^{2+}, Mg^{2+}, and Sr^{2+}. [c] Large-pore filter paper: 10-μm pore size poly(tetrafluoroethylene) filter paper. [d] Double-chain ammonium salt ($C_{16}N^+$ or $C_{18}N^+$) complexed with poly(styrenesulfonate). [e] Small-pore filter paper: 0.22-μm triacetylcellulose.

synthetic bilayer-forming material[124] have also been shown to produce oscillations when doped into the pores of a Millipore filter or a controlled-pore filter paper. A similar system, a Teflon filter doped with stearic acid and octanol, displayed excitability properties (membrane potential overshoot) upon a sudden pH change;[126] no oscillations in this particular system were reported, however. Some of the most recent papers report studies of oscillations that occur when these membranes are placed between NaCl and KCl solutions of identical concentration.[119,121-123,125] The goal in these experiments is to design a system that mimics the biological situation more closely by bathing the membrane with solutions typical of the inter- and extracellular media in biological systems. It is hoped that studies of these systems will lead to greater insight into the selectivity displayed by the biological systems. As in the investigations of lipid bilayers, it seems that oscillations and excitability can be achieved in a membrane that contains no channel-forming proteins at all. These results again point to the importance of the phase transition behavior of the lipids themselves in the excitability mechanism of biological membranes.

(3) *Langmuir–Blodgett Films.* A new technique for stabilizing lipid membranes was introduced by Yoshikawa and co-workers in 1985; in this technique, a Langmuir–Blodgett film of lipid is deposited onto a fine-pore membrane[127-129] (porous filter paper). The current–voltage characteristic of this type of membrane shows hysteresis, evidence of a negative conductance region; spontaneous oscillations also occur, so it appears that the essential features of the lipid membranes are preserved by employing this technique. As in the case of lipids and lipid analogues doped into pores of filters, the mechanism of oscillation appears to involve a phase transition of the lipid film.

(*b*) *Immobilized Enzymes.* As reviewed in the previous section, oscillations in an immobilized enzyme membrane system were first discovered[77] in 1973; in this system, the enzyme used was papain, a relatively unimportant enzyme in terms of its role in normal biochemical pathways. (However, papain is a fairly important enzyme for technological and biomedical uses.) Since the early 1980s, studies of oscillations in immobilized enzyme systems have dealt with the more biochemically relevant enzymes of acetylcholinesterase[130-132] (ACHE) and phosphofructokinase[133,134] (PFK), both important enzymes in the metabolic pathway. Recent experiments have added a fourth "enzyme" or complex, thylakoids,[135] to those previous studied; this complex structure is involved in the photosynthesis system of plants. The enzymes ACHE and PFK were immobilized in an inactive protein (albumin or gelatin, for ACHE and PFK, respectively) which was subsequently cross-linked with glutardialdehyde. Hervagault et al.[130,131] (1980) used a diffusion cell to study oscillations induced by the ACHE membrane which separated two flow compartments containing phosphate buffer; the substrate for the enzyme, acetylcholine (ACH), was injected into one compartment and the electrical potential across the membrane was monitored. Oscillations were observed over a range of ACH concentrations and flow rates. In a later experiment[132] (Friboulet and Thomas, 1982) it was found that the injection of an enzyme inhibitor, eserine, abolishes all oscillations, a result that was also observed when using a membrane without added enzyme. This confirms that the enzyme-catalyzed reaction is the source of oscillatory behavior in this system.

In another experiment[133] (Hervagault et al., 1983), an inert membrane separates a homogeneous solution containing the enzyme PFK from a homogeneous solution containing the enzyme substrate, NADH. The solutions are continuously flowing through the cell and oscillations in the concentration of NADH are observed spectrophotometrically. The authors have also carried out numerical studies[134] of a model of this system that generates even more exotic behavior than that observed experimentally, including sudden transitions between stable and periodic behavior.

A more recent study involves the immobilization of thylakoids[135] that have been extracted from green plants; thylakoids are an enzyme complex important in the photosynthesis pathway. The reaction that was mediated by the thylakoids in this experiment was the photochemical reduction of dichloroindophenol (DCIP). In this experiment, the thylakoids were immobilized in a thin membrane and affixed to the bottom of two identical reactors that were separated by an inert membrane. Buffered solutions containing the DCIP were pumped through the two reactors; one reactor was illuminated with light of wavelength 600 nm for the photochemical reduction. The solution pumped out of the other reactor was monitored for the reduced DCIP spectrophotometrically. An asymmetry of the obtained steady-state concentrations between compartments was observed along with hysteresis as a function of externally controllable parameters; no oscillations were reported, however.

(*c*) *Liquid Membranes.* Studies of variations of the original liquid membrane oscillator discovered by Dupeyrat and Nakache have appeared regularly in recent years. Some of these have produced extremely stable, periodic oscillations that persist for quite some time. This should be contrasted with the Dupeyrat–Nakache system, which displayed very noisy, not truly periodic oscillations. The new variations on the Dupeyrat–Nakache system, introduced mainly in Japan, have led to improvements in reproducibility and stability of oscillations.

The main constituents of a liquid membrane oscillator are two immiscible liquid phases (such as water and nitrobenzene): a surfactant molecule that exists alternately at different points in the oscillation as either a monolayer at the liquid interface or as micelles in the bulk; and a final species that is initially present in the liquid phase in which it is least soluble. This latter condition ensures that the system is far away from thermodynamic equilibrium, a condition necessary for the existence of any type of oscillatory instability. The membrane in these systems is the interfacial region between the aqueous phase and the organic phase and often involves a surfactant monolayer. It is somewhat different from the other membranes discussed in this review, all of which separate two aqueous phases. However, transport between the two phases in question (aqueous and organic, here) *does* occur and nonlinear phenomena, such as oscillations, are therefore possible.

Since 1983 a large variety of liquid membrane oscillators have been studied, particularly by Yoshikawa and co-workers. The first oscillations studied by this group[136] occurred in a system similar to that studied by Duperyat and Nakache in that it involved a liquid membrane formed in a beaker from two immiscible liquids. Yoshikawa and Matsubara found in 1983 that the frequency of the oscillations could be stabilized by replacing the organic solvent, which was nitroethane or nitrobenzene in the Dupeyrat–Nakache study, with nitropropane. Also, the addition of ethanol to the aqueous solution extended the range of concentration of surfactant over which oscillations could be observed. The second innovation from the Yoshikawa group also appeared in 1983 and consisted of a change in geometry.[137] The new geometry (see Figure 4), consisting of an organic layer in the bottom of a U-tube with two aqueous solutions on either side, has continued to be the configuration studied by this group. The left aqueous layer contains a surfactant and usually an alcohol, while the right aqueous layer is usually a salt solution or a sugar solution. The picric acid originally present in the organic phase of the Dupeyrat–Nakache system is replaced by 2,2′-bipyridine when an anionic surfactant is used in place of a cationic surfactant. Table III lists the types of liquid membranes[136–144] that have been shown to produce oscillations.

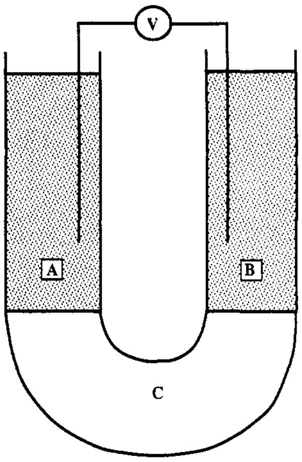

Figure 4. U-tube geometry utilized in recent studies of liquid membrane oscillators. Solutions A and B are aqueous electrolytes, one with added surfactant. These solutions may also contain a variety of additives such as alcohols or sugars. Solution C is an organic liquid that is immiscible with water; organic solvents used have included nitrobenzene, nitropropane, and nitroethane. The organic layer also includes a species such as picric acid or 2,2′-bipyridine that is more soluble in the aqueous layer. Mass transport of this species from the organic phase to the aqueous phase drives the oscillations. (Adapted from ref 151.)

TABLE III. Liquid Membrane Oscillators

cationic surfactant	ref
CTAC[a](aq)/PiH[b](nitroethane or nitrobenzene)	57
CTAB[c](aq), with or without ethanol/PiH(nitropropane)	136
CTAB(aq) + ethanol/PiH(nitrobenzene)/KCl(aq)	137
CTAB(aq) + ethanol/PiH(nitrobenzene)/sucrose(aq)	138, 139
D- or L-salt[d](aq) + ethanol/PiH(nitrobenzene)/D- or L-glucose(aq)	140
CTAB(aq) + ethanol/PiH(nitrobenzene)/optically active sugars[g]	141
anionic surfactant	ref
SDS[e](aq)/bpy[f] (80:20 nitrobenzene–alcohol)/NaCl(aq)	142, 143
Na oleate(aq) + alcohol/bpy(nitrobenzene)/NaCl(aq) or Na stearate	143
Na oleate(aq) + propanol + other[h]/bpy(nitrobenzene)/ NaCl(aq)	144

[a] Hexadecyltrimethylammonium chloride. [b] Picric acid. [c] Hexadecyltrimethylammonium bromide. [d] (α-Methylbenzyl)dimethylmyristylammonium bromide. [e] Sodium dodecyl sulfate. [f] 2,2′-Bipyridine. [g] D-Glucose, -fructose, -galactose, -sorbitol, -mannitol, -ribose, -xylose, -sucrose, and -maltose; L-sorbose and -arabinose; and glycine. [h] NaCl, sucrose, HCl or quinine chloride.

The mechanism thought to be responsible for the oscillations in these new systems as well as those observed by Dupeyrat and Nakache involves the periodic formation and disruption of a monolayer of surfactant molecules at the water–organic interface. The surfactant forms a complex with the species which is present out-of-equilibrium (such as the alcohol in the Yoshikawa experiments). When a critical monolayer coverage is reached, the surfactant–alcohol complexes are abruptly transferred across the interface into the organic phase, where the surfactant then forms inverted micelles in the bulk. The phase transition process, monolayer → micelles, is the key step in this mechanism and the transfer of alcohol is the driving force.

3. *Theoretical Studies*

(*a*) *Immobilized Enzymes.* A general feature of most mechanisms that result in oscillations are coupled positive and negative feedback steps. A good example of this occurs in membranes containing immobilized enzymes. In these systems, the positive feedback is associated with the enzyme-catalyzed reaction, while the negative feedback is due to the mass transport of substrate to the enzyme location. The latter step often occurs by diffusion through the membrane matrix and is quite slow.

One of the early theoretical studies (discussed in the previous section) of oscillations in immobilized enzyme membranes was carried out by Zabusky and Hardin[78] (1973). Dai[145] (1980) continued this work by studying a zero-dimensional (i.e., no spatial dependence) approximation of the reaction diffusion equations for the enzyme-catayzed hydrolysis of a generalized substrate. The zero-dimensional approximation reduces the model to a pair of coupled, nonlinear ordinary differential equations rather than partial differential equations. Dai was able to find a sufficient condition for the existence of a limit cycle solution to this pair of equations.

Chay[146] (1980) also derived a model for hydrolysis via an immobilized enzyme catalyst, but her approach used a more microscopic approach, that of absolute rate theory. However, the microscopic model was found to be equivalent to a macroscopic one (mass action reaction diffusion, in which activation barriers do not appear explicitly) under certain conditions involving the presence of a buffer. The specific case considered was papain enzyme immobilized in bovine serum albumin; experimental observation of pH oscillations in this system had been previously reported by Naparstek, Thomas, and Caplan[56] in 1973. The model predicted the observed pH oscillations and revealed that they were related to a synchronous oscillation in buffer penetration depth. A few years later, Chay and Zabusky[147] (1983) applied the same multibarrier model to acetylcholinesterase immobilized in a bovine serum albumin membrane. In this simulation, single-frequency oscillations were observed if salt was not added to the system; this observation is consistent with the experimental observations of Friboulet and Thomas.[132] Interesting bursting-type oscillations involving more than one frequency were observed in the presence of added salt; this may have implications for the possibility of chaotic behavior in these systems (see section II.B.4).

Kernevez and co-workers have also published several modeling studies of immobilized enzyme membranes, primarily involving numerical simulations. In 1983, Kernevez et al. published[148] a numerical study of a model of phosphofructokinase immobilized in a membrane and observed oscillations similar to those seen experimentally. The numerical method used in this study involved a continuation technique (AUTO, available from E. Doedel at the CalTech Applied Math Department) for tracing out the periodic solutions to a system of ordinary differential equations. This numerical method is different from but complementary to the usual numerical approach for solving systems of ordinary differential equations, such as Runge-Kutta or Gear's algorithm. Kernevez, Doedel, and Thomas published a recent review[149] (1985) describing the various modeling studies carried out by themselves and co-workers since 1970. Application of AUTO to single and multiple steady-state systems, as well as oscillatory systems, is described in this review.

(*b*) *Liquid Membranes.* Yoshikawa and Matsubara (1983) published a two-species model[136] for the oscillations observed in the liquid membrane system studied experimentally by their group. The model consisted of two pairs of coupled ordinary differential equations, each pair applying over different intervals of time. The two time-dependent species in the model were the cationic surfactant and a small anion, consistent with the important species in the mechanism originally proposed by Dupeyrat and Nakache[57] (1978). However, Yoshikawa and Matsubara's model includes explicit nonlinear terms to model the cooperative effects, terms that were left out by Dupeyrat and Nakache but that Sanfeld et al.[150] claimed were necessary for the existence of oscillations. The model of Yoshikawa and Matsubara[136] reproduces the qualitative features of the observed oscillations but is partially ad hoc in nature; in addition, the model is quite unusual in the sense that it consists of two sets of differential equations that apply over different phases of the oscillations. This "piecewise" feature makes the theoretical validity of the model somewhat questionable.

In later papers, the model described above was improved by introducing a third variable, the ion-pair complex, and eliminating the questionable "piecewise" nature of the equations. Toko et al. (1985) introduced[139] this modified model and confirmed the existence of simple, single-frequency oscillations via numerical studies with it. They also performed a stability analysis of the steady states of this model, showing the existence of a Hopf bifurcation at parameter values where oscillations begin to be observed numerically.

Yoshikawa, Maeda, and Kawakami (1988) recently reported[144] complex, two-frequency, bursting-type oscillations in a slightly modified version of this model. The modification involves a slight variation in the nonlinear term in the rate equation for the ion-pair complex. The nonlinearity is introduced in this work as well as in the earlier paper by Toko et al.[139] (1985) via mathematical arguments that an N-shaped nonlinear function is necessary for the existence of oscillatory behavior. Yoshikawa et al. (1988) argue[144] that this can be justified by considering the form of the necessary electrostatic term (from Debye–Hückel theory, for example) in a virial expansion for the interfacial pressure. Such a term has the requisite N-shaped form, but the relationship between interfacial pressure and ion-pair complex surface coverage is not explicitly described. Nevertheless, this latter model is the most complete of those studied to date for liquid membrane oscillators and deserves to be investigated more fully. In particular, the model should be more rigorously derived, especially with regard to the expected physical and chemical phenomena occurring in the system. Yoshikawa has reviewed the theoretical studies[151] of this type as well as experimental evidence supporting this interpretation.

Although it is somewhat outside the scope of this review, a fair amount of interest exists in the coupling that can occur between surface tension, other hydrodynamic features, and interfacial chemical reactions.[152-158] One application of this type of mechanism other than to the liquid membrane oscillators reviewed here[156,157] is to the phenomenon of cell motility over surfaces.[158]

(*c*) *Negative Conductance.* A plot of current vs voltage (I–V) would be linear if Ohm's law were obeyed.

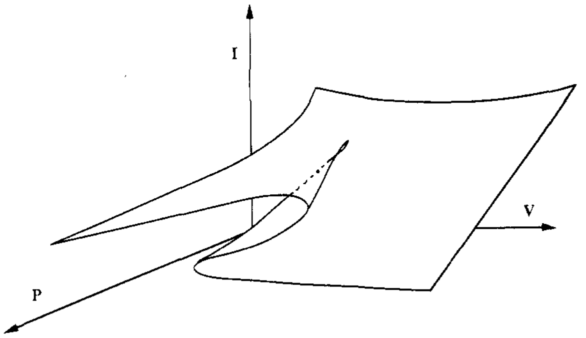

Figure 5. Folded surface showing the dependence of current (I) on voltage (V) and pressure (P) in the Teorell oscillator (adapted from ref 22). At high pressures a region of negative conductance exists; this corresponds to the middle sheet of the folded surface. At low pressures, no fold exists and oscillations are not possible. In other membrane oscillators some other parameter may play the role of the pressure.

The slope of such a plot is the conductance in this linear case. Membranes and other electrochemical and electrical systems often display highly nonlinear $I-V$ curves, including plateaus, i.e., regions of zero conductance, and even regions of negative conductance. The latter characteristic has long been known to be associated with excitability in nerve membranes and other excitable tissues.[159-164]

As Teorell pointed out[23] in 1962, the occurrence of a region of negative conductance is an indication that the system is unstable and might display this instability in the form of oscillations. Even at this early date, it was known that tunnel diodes possessed current-voltage characteristics with negative conductance regions and were also unstable and excitable. In developing a model of the charged glass membrane oscillator he studied, Teorell found[21] that a third variable, the hydrostatic or osmotic pressure, was needed to explain the oscillations. In the model the pressure functions as a control parameter, taking the system back and forth through a negative conductance region, as shown in Figure 2. A plot of current vs the two variables voltage and pressure for Teorell's electrohydraulic model of a glass membrane oscillator is shown schematically in Figure 5. It can be seen that at high pressures a region of negative conductance exists, while at low pressures it does not. When the pressure is swept past the value at which the surface becomes folded, oscillations become possible. This explanation can be generalized to other systems in which some other variable plays the role of the pressure.

The self-generated and -sustained oscillations observed in DOPH-doped Millipore filters are associated with $I-V$ curves that display hysteresis,[165] as would be expected to occur if a region of negative conductance existed. The negative conductance region corresponds to an unstable state, so variations in a control parameter will cause transitions to occur at different values of that parameter. The origin of the negative conductance region and the associated hysteresis is thought to be related to the phase transition,[166] which occurs in conjunction with the observed oscillation.

The physical or chemical origin of a region of negative conductance may vary from one system to the next. In electrochemical reactions, usually involving reduction of a metal surface, the phenomenon that leads to negative conductance is known as passivation. The physical process associated with passivation is thought to involve formation of an oxide film, but even this is not generally accepted. In addition, the mechanism by which such film formation leads to a region of negative conductance is also not well understood.

A region of negative conductance in the $I-V$ characteristic of both electrochemical and membrane systems has been implicated in the mechanism by which electrical oscillations arise in these systems. The $I-V$ curve may be time dependent, but if we consider the steady-state case, oscillations may be explained as arising from bistability associated with a hysteresis loop. Degn[167] has proposed a mathematical model for the origin of electrochemical oscillations based on this idea. As Degn pointed out, the negative conductance region of the steady-state $I-V$ curve plays a major role in the mechanism of the origin of oscillations but must be accompanied by some time-dependent process to drive the system around the hysteresis loop. Degn's theory suggests that time-dependent concentration polarization is involved in the electrochemical oscillations. It is not yet clear what process plays this role in membrane oscillators, although the two types of systems are very similar. Negative conductance in electrochemical systems has been associated with oscillations;[168-172] recent studies have focused on chaotic oscillations in these systems which appear to be of the "bursting" type.[173-179]

(*d*) *Instabilities in the Nernst-Planck Equation.* The existence of oscillatory behavior in some membrane systems may be related to an oscillatory instability which can be shown to occur in the Nernst-Planck flux equations when applied to ion-exchange membranes. The $I-V$ curves for these membranes consist of a linear ohmic region followed by a plateau or "limiting" current. In 1979 Rubenstein and Shtilman showed[180] that this characteristic shape was not due to concentration polarization effects, as had been previously postulated. If concentration polarization were occurring, the plateau would represent the asymptotic, high-V behavior. However, in cation-exchange membranes, it is found that the plateau is followed by another region of rapid current increase, or "overlimiting" current. In this region, large fluctuations in current readings are observed; the fluctuations are often of the same order of magnitude as the current itself. Hydrodynamic turbulence has been proposed as a cause of these fluctuations, but a source due to transport processes is more likely.[181]

Rubinstein and Segel (1979) showed[182] that steady-state solutions to the Nernst-Planck equation become unstable if electroneutrality is not imposed on the electrolyte solution in the region near the membrane-electrolyte interface. The lack of electroneutrality in this unstirred layer introduces a small parameter in the Poisson equation for the electrical potential. Rubinstein and Segel used this small parameter to carry out an asymptotic expansion of the Nernst-Planck-Poisson system of equations and to show that the steady-state solutions of the first terms in the expansion were unstable to time-dependent perturbations. In 1981, Rubinstein[183] performed a similar analysis of the full Nernst-Planck-Poisson system without resorting to an expansion and found a similar result. In 1984, he showed[184] that the instability was restricted to a certain class of boundary conditions on the equations. Furthermore, it was discovered that the instability *was* associated with large current fluctuations but also with an unbounded accumulation of salt in the unstirred layer. This result indicates that some mechanistic aspect is missing from the model, one that would serve to remove the accumulated salt. Rubinstein also mentions that oscillatory behavior is a possible manifestation of the instability, although no oscillatory solutions of the model equations are reported. Possibly, the missing aspect that prevents unbounded salt accumulation might also lead to oscillatory behavior. Some related recent work by Rubinstein and co-workers[185,186] involves the imposition of high-frequency voltage modulations on an ion-exchange membrane system. An increase in the dc component of the responding current is observed both experimentally and theoretically. This result is thought to be due to the unstirred layer acting as a nonlinear amplifier of the imposed current fluctuations. A footnote in these latter papers indicates that measurements in the presence of periodic bulk flow, e.g., electroosmosis, was also investigated and will be reported in a forthcoming paper. These results should be particularly interesting and applicable to the Teorell-type membrane oscillators in which periodic bulk flow of solution through the membrane occurs in synchrony with the voltage oscillations.

(*e*) *Role of Phase Transitions.* Lipids or lipid-type molecules may exist in a variety of different phases including bilayers, multilayers, micelles, large vesicles, and disordered phases. Transitions between these phases may play an important role in the mechanism of oscillation in lipid bilayer systems. Toko and co-workers[165] have demonstrated experimentally that membrane voltage oscillations across Millipore filters doped with lipids or lipid analogues are associated with periodic phase transitions of the lipid material. These phase transitions are detected by differential scanning calorimetry or similar techniques and are found to occur in synchrony, i.e., with the same period, as the observed voltage oscillations. The phase transition may change the permeability of the bilayer, allowing ions to traverse the membrane at a different rate; this could lead to a short-lived surge in current and voltage. Evidence for this effect was provided by Okahata and En-na,[187] who demonstrated that the permeability of a Langmuir–Blodgett monolayer could be controlled by varying the phase of the monolayer from a solid phase to a liquid crystalline phase.

In part because of these explicit observations in artificial systems, several investigators have proposed that phase transitions might be involved in the mechanism of excitability in biological membranes. Lahiri, Goswami, and Dasgupta[111] have suggested that the membrane potential depolarization wave is propagated along the nerve axon by a physical wave associated with a lipid bilayer phase transition. Their theory is based on the observation of Cotterill[72] that the melting point, i.e., phase transition temperature, of the bilayer is strongly influenced by the electric field strength. Recently, Eisenberg and Grodzinsky[188] have shown that the permeability of an ion-exchange membrane is also influenced by changes in applied electric field strength. Cotterill has suggested[112] that the nerve impulse might involve a localized phase transition in the region of the bilayer just adjacent to the channel proteins. In this scenario, it is the lipid matrix, not the channel proteins themselves, that is the source of the observed action potential. Further evidence for the role of local phase transitions near and around ion channels has been demonstrated by Lee,[74] who showed that the addition of local anesthetics to nerve membranes containing sodium channels triggers a localized melting of the lipid bilayer. Finally, the coupling of hydrodynamic bending modes to transmembrane fields has been studied theoretically by Wendel, Bisch, and Gallez.[189-191]

Another type of phase transition that may play a role in excitability phenomena is that of lateral phase separation.[192-197] The bilayer in cell membranes is composed of a mixture of a large number, dozens, of different phospholipid molecules. These different molecules are not entirely soluble in one another and might be expected to undergo some degree of lateral phase separation or clustering. As Sackmann has pointed out,[194] lateral phase separation will lead to an asymmetry of the bilayer and cause a local spontaneous curvature which would be associated with a small spontaneous electric polarization, i.e., a localized increase in membrane potential. This may, in turn, be coupled to variations in transmembrane concentration gradients.[197] The coupling between membrane potential, lateral phase separation, and local curvature of the bilayer may be quite complex. The types of phase separations that could occur might also be very complex, as McConnell and co-workers have shown.[198-204] Monolayers composed of a single type of phospholipid plus a small percentage of cholesterol form plastic crystals, dispersed throughout a disordered liquid phase. Even equilibrium structures (i.e., those which form and dissipate reversibly) are highly ordered and intricate, consisting of symmetric spirals and branched structures.[202] Nonequilibrium (irreversibly formed) structures may be even more complicated and have been shown to possess fractal characteristics.[205] It is clear that the phenomena of oscillations and excitability in membranes is intimately connected to and cannot be divorced from the phenoma of spatial nonuniformities. The latter will be reviewed in section III.

4. Chaos

(*a*) *Biological Systems.* As in the preceding decade, studies of chaotic behavior in biological membranes have primarily involved perturbing spontaneously oscillatory systems with periodic forcing sources. In many cases, these systems are neurons and the intent of the studies is to mimic the response of the neuron to the periodic bursting behavior of its neighbors to which it is synaptically connected. The action of even a simple sinusoidal perturbation on most neurons leads to very complex responses including quasiperiodicity, phase-locking, and chaotic behavior.

Guttman et al.[206] (1980) carried out pioneering work on the sinusoidal forcing of the squid giant axon, the most popular neuronal system for study. These investigators observed entrainment of the natural oscillations in the axon to the frequency of the sinusoidal perturbation. Subsequent work by Matsumoto et al.[99] (1984) confirmed the entrainment response of this system and showed that quasiperiodicity and chaos were also possible response modes. Aihara et al.[207,208] (1985, 1986) extended their work by varying the frequency of the applied sinusoidal current; an alternating sequence of periodic and chaotic states was observed over the interval of frequencies studied. In these experiments, the axon is prepared in a spontaneously oscillatory state by placing it in a 1:9 mixture of artificial seawater and 550 mM NaCl. The sinusoidal perturbation is usually a modulated current imposed via a platinum wire inserted directly into the interior of the axon.

Neurons from other organisms have also been used in studies of the chaotic response. Holden and coworkers have concentrated on the giant neuron of the pond snail.[100–103] In their initial 1981 study, Holden and Ramadan[100] applied a constant (not periodic) current to the neuron and observed a periodic (single frequency) response at low current magnitude; at higher currents, the response was characterized by multiple frequencies and is of the bursting type, i.e., some number of small-amplitude oscillations followed by a sudden "burst" of large-amplitude oscillations. A companion study[101] involved the imposition of sinusoidally modulated current. The neurons displayed both transient and periodic responses. As the frequency of the current was varied, one interesting type of response was observed: in narrow intervals of the forcing frequency it was found that the system response involved "stochastic switching between simple entrained patterns". The more usual response that was observed was phase-locking into simple entrained, periodic patterns. The type of chaotic behavior that involves stochastic, or random, switching between simpler periodic states is common in systems in which the chaos arises via coupling of oscillators.

Holden, Haydon, and Winlow (1983) observed[209] a similar chaotic response to the imposition of *constant* current if the neuron was placed in a solution with low Ca^{2+} concentration. Similarly, the addition of menthol (an anesthetic) to the low-$[Ca^{2+}]$ solution produced the same response. Menthol and low $[Ca^{2+}]$ evidently interfere with ion transport through the neuronal membrane, causing a change in the current flowing into the cell. In similar experiments,[102] the snail neuron was found to display complex bursting patterns in the presence of 4-aminopyridine (4AP), which blocks voltage-dependent K^+ channels. Several different types of voltage-dependent K^+ channels exist in molluscan membranes, so it is uncertain which blockage is the source of the response; it is important to note that the blockage will increase the inward current, though, so it may be that the response is not specific to the particular ion channel involved. A similar response is seen[103] when tetraethylammonium ion (TEA^+) is iontophoretically injected into the neuron. This species also blocks K^+ channels; in this experiment, the TEA^+ is carried into the cell by a current of about 2 nA. The response in this particular experiment is, therefore, due to *both* the iontophoretic current and the blockage of the K^+ current.

Hayashi et al.[107,108] (1982, 1983) studied the response of a freshwater algae internodal cell to sinusoidal current stimulation. The giant internodal cell (cylindrical, ~300-μm diameter, ~2 cm long) of *Nitella* can be induced to fire spontaneously if placed in artificial pond water with 30–60 mM NaCl added. The imposition of sinusoidal current results in entrainment of the spontaneous oscillations as well as quasiperiodicity and chaos, as in the studies of the neuronal systems. This group has also studied different neuronal systems, both the "silent"[210] (not spontaneously bursting) and pacemaker[211] neurons of the mollusc *Onchidium*. The imposition of sinusoidal current to either of these neurons produces responses similar to those observed in squid giant axon and the pond snail neuron, namely, phase-locking, quasiperiodicity, and chaos.

(*b*) *Artificial Systems*. Complex oscillatory behavior indicative of the possibility of chaos has been observed in several artificial membrane systems. Earlier work by Arisawa and Furukawa[55] (1977), discussed in section II.A, involved the additional feature of an imposed pressure gradient. This resulted in higher frequency oscillations, but of the type we would now characterize as "bursting" (more than one frequency) if large amounts of DOPH were used. Toko et al.[116] continued studies of the DOPH-doped Millipore filter system and observed that extremely long (~1 h) periods of quiescence were interrupted by sudden, large spike-type oscillations in the potential when the membrane was placed between two solutions of KCl of differing concentration (one 5 mM, the other 100 mM). In a 1984 paper, Toko et al. proposed[117] a theoretical model for the bursting behavior in the DOPH system. The model is complicated, specific for the DOPH system, and includes terms that describe the phase transition kinetics known to occur in the DOPH molecules within the pore. A subsequent study by this group[119] involving the imposition of a direct current on the membrane, showed that possible chaotic behavior can result. The authors suggest that the existence of two frequencies can be explained in terms of a nonlinear coupling between a high-frequency mode involving DOPH molecules adsorbed to the surface of the filter and a low-frequency mode involving a phase transition of the molecules in the pore.

Recent work on liquid membranes has revealed evidence that multifrequency oscillations and chaos may also exist in these systems. A particularly clear example was observed by Yoshikawa and Ishii[123] (1986) for a U-tube arrangement involving immiscible water and nitrobenzene solutions. If 2,2′-bipyridine is present in the nitrobenzene phase and sodium oleate, NaCl, and 5% butanol are present in the water phase, complex oscillations consisting of large-amplitude, low-frequency spikes superimposed on small-amplitude, high-frequency oscillations are observed. A similar result is seen when the butanol is replaced with 10% propanol and sucrose (Yoshida et al.,[143] 1988). In this latter work, a model is proposed and numerical studies with this model reproduce the two-frequency mode. The model involves the formation of complexes at the liquid–liquid interface and an N-shaped nonlinear isotherm for the interfacial pressure. It is interesting to note that the investigators find that the use of shorter chain alcohols never leads to bursting behavior. An explanation for this behavior may be forthcoming from future theoretical studies.

(*c*) *Theoretical Studies*. Most of the theoretical work regarding chaotic behavior in membrane systems has focused on well-established models of neurons or bursting cells. By far the most frequently studied model for neurons, particularly applicable to the squid giant axon, is the Hodgkin–Huxley (HH) equations.

Hassard[212] showed a decade ago that the HH system of equations possesses a Hopf bifurcation and can sustain stable periodic solutions. Rinzel and Miller[213] studied the HH system numerically and found unstable periodic solutions as well. Aihara and Matsumoto[214] showed that these equations could also display bistability. Labouriau studied the bifurcation behavior of the HH equations and found evidence for chaotic behavior in the form of homoclinic orbits.[215,216] By applying a sinusoidal forcing term to the equations (to model the experimental application of a sinusoidal current), many investigators[217-221] were able to reproduce the chaotic response seen in the actual neurons.

Other models of neuronal[222] or pacemaker[223-226] activity have been studied and found to generate chaos even without forcing. In addition, the newly developed mathematical techniques of analyzing experimental data have led to the characterization of the chaotic behavior seen in neuronal systems in terms of Lyapounov exponents, fractal dimensions, power spectra, and the like.[207,208,227-229] These techniques do not seem to have yet been utilized to study the suspected chaotic behavior in artificial membrane systems, though.

III. *Spatial Nonuniformities in Membranes*

A. Historical Review

Some of the recent interest in biological pattern formation was sparked by the discovery of spontaneous spatiotemporal pattern formation[230] in a nonliving system, the Belousov–Zhabotinskii (BZ) reaction. Slowly moving chemical waves form when the reaction mixture is spread in a thin layer and left unstirred. This arrangement is geometrically similar to a membrane but differs in that the thin layer is not a barrier between two bulk solutions. One important feature of a membrane that does not exist in a system with this geometry, then, is the capability to transport chemical species across the membrane or the possibility of imposing perpendicular driving forces (electrical or otherwise) on the thin layer or membrane. Since the original discovery of chemical waves in the BZ reaction, similar spatiotemporal structures have been found in thin layers of other reacting mixtures, including enzyme reactions, such as glycolysis (see Müller et al.[231]). Avnir and Kagan[232] also observed patterns at a liquid–liquid interface; these patterns were not waves but stationary patterns (i.e., standing waves) possibly stabilized by evaporation and convection. Because of the important differences between these thin-layer systems and membranes, discussed above, the thin-layer systems will not be considered further. However, one study in which a membrane actually *was* used in a similar system was reported by DeSimone, Beil, and Scriven[233] in 1973. They observed chemical waves in a BZ reaction system in which the ferroin indicator had been immobilized in a collodion gel membrane. Slowly moving chemical waves, unperturbed by convective effects, were observed. A similar result involving a continuous-flow unstirred reactor was recently reported.[234] Again, transport perpendicular to the plane of the thin layer or membrane cannot take place in either of these systems, however, so they differ in an inherent and important way from the other membrane systems reviewed here.

1. Biological Systems

As early as the 1940s the importance of electric fields in development and growth processes had been stressed (Lund,[235] 1947). The existence of an electric field implies a patterned, or spatially nonuniform, electrical potential, which may or may not be the result of a self-organization phenomenon, such as the reaction–diffusion mechanisms discussed below. A more recent example is the spatially nonuniform membrane potential observed in the giant cylindrical alga *Acetabularia* by Novak and Bentrup[236] in the 1970s. A similar phenomenon in a system with spherical symmetry was observed in the eggs of the seaweed *Fucus*[237] and *Pelvetia*[238] by Jaffe and co-workers. Here, the establishment of the pattern in electrical potential was found to be the essential first step in the morphogenetic process that takes the egg cell from its undifferentiated single-cell state to a differentiated multicellular organism.

The slime mold *Dictyostelium discoideum* is a widely studied multicellular system which exists in a patterned state under starvation conditions. The mold is a single-celled organism if food is plentiful, but upon starvation, organizing centers develop that send out chemical signals that cause aggregation of the cells. The aggregation phenomenon is essentially driven by a chemical wave and results in the formation of a multicellular mold organism. This multicellular organism, a fruiting body, produces spores that are presumably used to spread the mold to a region where food is more plentiful. *Dictyostelium* has provided an excellent example of the phenomenon of pattern formation in biological systems, and many papers have been written about its development process.[239]

2. Artificial Systems

As mentioned above, much of the recent interest in pattern formation in biology was the result of the discovery of spatial patterns, including waves, in purely chemical systems. The study of pattern formation phenomena in artificial membrane systems has not been very extensive, and the only experimental example of spatial patterns due to reaction–diffusion-type instabilities in artificial membranes is the study of the BZ reaction in a collodion membrane (DeSimone et al.,[233] 1973) mentioned above. No studies of the effects of these patterns on transmembrane transport in this system were reported, however.

Transport through a membrane deliberately constructed to be nonuniform was, however, studied and found to be enhanced by the nonuniformity. Weinstein and Caplan[240] (1968) studied salt transport through charge mosaic membranes made by arranging a single layer of cation- and anion-exchange beads in a checkerboard pattern within an impermeable matrix. The juxtaposition of the oppositely charged beads leads to small current loops that tend to enhance the rate of transport of salt through the composite membranes. A tenfold increase in KCl permeability was observed over that of control membranes composed of just the cation-exchange or just the anion-exchange beads.

An interesting class of membranes made of polymers with a hydrophobic backbone and ionic pendant side groups placed at regular or irregular intervals along the chain provide another example of enhancement of transport by spatial nonuniformity. This type of material has come to be known as an "ionomer" since the membranes that are made from the material have a fixed charge due to the ionic side groups. In 1968, Longworth and Vaughan[241] showed that ionomer membranes were nonuniform due to the clustering of the side chains into small (~40-Å diameter) globules interspersed through the hydrocarbon matrix formed by the backbone chains. In 1977, Yeo and Eisenberg[242] discussed evidence for a clustered supermolecular structure of a very important member of this class of ionomers, a perfluorosulfonic acid polymer manufactured by Du Pont with the trade name Nafion. This particular material has become very important in the field of electroanalytical chemistry as a polymer coating for electrodes. More recent studies, including those which show that this material has enhanced transport properties, possibly due to its nonuniform structure, will be discussed in section III.B.

3. Theoretical Studies

(*a*) *Self-Organization/Pattern Formation.* In 1952, Turing[243] published what came to be an extremely influential paper proposing a chemical basis for the phenomenon of morphogenesis, i.e., the generation of form in a developing biological system. Turing argued that the ordered patterns of differentiated cells that arise in developing and mature organisms could be explained via a chemical mechanism that did not involve the details of the gene transcription process that initiates the differentiation. His model is based on the discovery that a spatially uniform state of a reacting mixture, such as might exist in a field of cells in an embryo, can be unstable to spatially nonuniform perturbations if appropriate interactions between chemical reaction and transport occur. Although some of the precise details of Turing's model were later criticized as not totally correct or representative of actual developing systems, the basic idea has remained a firm basis for most subsequent theoretical work in the biological development field as well as the study of pattern formation in nonliving systems.

In the 1960s and 1970s, Turing's work was "rediscovered" when it was found that spatiotemporal structures were, indeed, observed in purely chemical systems. The most widely studied system, the Belousov–Zhabotinskii reaction, produces spiral waves when the reaction mixture is spread, unstirred, in a petri dish. An important distinction between these waves and the patterns envisioned by Turing were that the latter were stationary structures, not moving waves. The older experiments of Liesegang,[244] who discovered alternating precipitation bands in a system in which mass transport was coupled with the precipitation chemical reaction, also were "rediscovered" and studied again as possible physical examples of the Turing phenomenon.

These experimental discoveries encouraged further theoretical work on the phenomenon of pattern formation in chemically reacting systems, particularly those with biological relevance. In 1966, Gmitro and Scriven[245] published an influential paper that derived the mathematical requirements for the generation of spatial patterns in a chemically reacting system. This work was expanded upon by Othmer and Scriven[246] (1969). About the same time (1969), Wolpert[247] published a theory of pattern formation in biology that focused on the idea of positional information. According to Wolpert's theory, every cell in a developing biological system possesses information with regard to its ultimate position in the mature organism; this information may exist in the form of a "morphogen", i.e., a chemical substance that is nonuniformly distributed and whose gradient would provide a vector pointing the direction to the ultimate position of the cells in the developing organism. Goodwin and Cohen[248] (1969) proposed a model to explain the origin of the positional information map, i.e., the nonuniform distribution of morphogen. The model relies on wavelike propagation of activity over a field of cells, emanating from oscillatory pacemaker centers. This model is similar to that of Turing but includes the aspect of regulation, an important biological property necessary for the existence of *stable* patterns.

These theories of biological development were controversial at the time of their proposal and, indeed, remain controversial even today in many aspects. Their introduction, however, stimulated a fair amount of theoretical and computational work involving numerical simulations of biological development processes, some of which were quite successful in reproducing pigmentation patterns, limb formation patterns, etc. (see, for example, Gierer and Meinhardt,[249–251] Martinez,[252] Lacalli and Harrison,[253] and Murray[254]). Some theoretical simulations have focused on the importance of cell–cell interactions in the patterning mechanism (Babloyantz[255]), and a fair amount of work utilizing the ideas of cellular automata[252] has appeared in recent years.

At the same time that the reaction–diffusion theories of morphogenesis were being developed, other investigators were studying strikingly similar phenomena occurring in nonliving systems; the most widely studied of these were the Belousov–Zhabotinskii patterns and Liesegang rings. Some of the theoretical investigations that were important in early years include those by Nicolis and Auchmuty,[256,257] Kopell and Howard,[258] Winfree,[259] Kubicek et al.,[260] Othmer,[261] and Nazarea.[262] These studies served to determine the variety of allowed patterns for chemically reasonable reaction kinetics and transport processes.

(*b*) *Transport in Nonuniform Systems.* Early theories of transport in nonuniform systems were rare because these systems are difficult to model without the most sophisticated, modern techniques, which usually require extensive computer simulations. Glueckauf[263] (1962) put forward one of the few such theories as a means of explaining unusual observations of transport in ion exchangers. The data were consistent with a network type of nonuniformity, i.e., interconnected fissures or channels, filled with electrolyte, in which the transport of salt was assumed to occur. The theory involved the assumption of a Donnan equilibrium for a representative small volume element in the system; it is semiempirical and relies on the inclusion of some experimental data.

The possibility that nonuniform systems might possess unique or unusual transport properties was pioneered by Sollner. As early as 1932, he predicted[264] that a mosaic of cation- and anion-selective elements would exhibit unique transport properties involving small, localized current loops; Weinstein and Caplan were able to experimentally demonstrate[240] this effect in 1968 (see section III.A.2). In a related example, Cantril and Pohl[265] (1968) reported the phenomenon of "insulator-induced conduction" in which the presence of insulating material near or around a dielectric serves to focus and enhance the current through the dielectric. The ion-exchange membranes studied by Glueckauf[263] could be affected by this phenomenon since the network of fissures constitutes a dielectric phase that is surrounded by a nonconductive matrix. Recent evidence regarding the structure of ionomers[266] shows that these materials are microheterogeneous in structure, with conducting regions interspersed through insulating fluorocarbon regions; these might constitute another system in which the phenomenon of insulator-induced conduction might be operative since it is known that some ionomers display enhanced electrical conduction properties.

B. Recent Studies (1979–1989)

1. Biological Systems

Chara, an aquatic plant, develops a banded pattern of alternating acid and alkaline regions on its internode, a single cylindrical cell.[96,267–270] This banded pattern has been found to be associated with a variation in membrane potential along the length of the cell. The bands appear in response to sudden exposure to light and other disturbances. Extracellular currents are found to circulate from the alkaline to the acid regions in roll-like patterns. Similar band-type distributions of chloroplasts in the green alga *Acetabularia*, a cylindrical single-celled organism, have been found to develop when the extracellular Ca^{2+} concentration is varied. The cylindrical geometries of the internodal cell of *Chara* and of *Acetabularia* seem to be the most important factor in the determination of the stable patterns that develop in these systems.

A similar phenomenon is observed in a multicellular system, root sprouts of the azuki bean (*Phaseolus chrysanthos*) (see Toko et al.,[271] 1987). The electrical potential near the surface of the root develops a banded pattern with a wavelength of about 2 cm as the root grows. This is associated with an inhomogeneous distribution of ATP content and enzymic activity along the length of the root. The speed of growth of the root is extremely sensitive to the presence of an applied electrical disturbance. It is quite likely that this spatial patterning phenomenon is related to the current and voltage oscillations observed near sprouting bean roots over 30 years ago[8–14] (see section II.A) although this possibility has not been investigated in depth.

Nuccitelli[272] (1988) recently reviewed a whole host of phenomena observed in growing and developing biological systems that may be due to electrical nonuniformities possibly arising from reaction–diffusion mechanisms. Ionic currents have been found to exist near many developing embryonic systems (of which sprouting beans is an example from the plant world) as well as near the site of growth in healing wounds and regenerating limbs, etc. These currents are necessarily associated with nonuniform electrical potentials, since a current implies a nonzero electric field, which implies a gradient in the potential. Examples of these currents have been found in systems ranging from pollen grains and fungus to carrot and tobacco embryos to regenerating limbs in amphibians and wounded epithelial cells and skeletal muscle. Nuccitelli emphasizes the role of Ca^{2+} as a possible "morphogen" that could be moved by these currents. [As discussed in section V.B, however, this identification of Ca^{2+} as the morphogen does not address the interesting and important question of the physicochemical origin of the currents and the patterned electrical potentials.] The calcium ion is a particularly important ion in development partly because it directly influences the membrane's permeability to other ions and could have an effect on the generation of the currents since the cell membranes are undoubtedly involved in the current generation process. The reader is referred to Nuccitelli's excellent review for further details regarding electrical patterns in biological systems.

2. Artificial Systems

(*a*) *Electrochemiluminescence*. An interesting recent example of an artificial system that exhibits patterned distributions is an electrochemiluminescence cell[273] (see Schaper and Schnedler, 1982). These cells are constructed of two plane, polished electrodes separated by a distance of only 60 μm. The space between the electrodes is not filled with an electrolyte but rather with solutions of neutral species. Even in these electrolyte-free media, small concentrations of ionic impurities exist, and these tend to build up in thin ionic layers near the electrode surface. In the particular system studied, a voltage is applied to the electrodes. It appears that free radicals are formed which are then transported across the small spacing by a mechanism that is predominantly hydrodynamic. Bēnard-like cells appear and hexagonal luminescent structures can be seen due to the luminescence that occurs upon radical recombination combined with the hydrodynamic transport process. An interesting result is that a higher current efficiency is achieved when one of the electrodes is deliberately roughened with scratches whose spacing is on the order of the size of the Bēnard cells. The current efficiency is nearly 3 times as large under these conditions.

(*b*) *Phospholipid Monolayers*. McConnell and coworkers have studied a number of different examples of pattern formation in phospholipid phases, particularly monolayers.[198–204] One interest of this group has been in explaining the origin of regular, spatially periodic structures observed in freeze–fracture electron micrographs of dimyristoylphosphatidylcholine vesicles;[198] these periodic structures, or corrugations, seem to be associated with fast diffusion phenomena.[192] The origin of these bands is intrinsically interesting but they have also proven quite useful for correlating data obtained from the vesicles via fluorimetry, calorimetry, etc. One theoretical explanation advanced by this group is that the properties of the $P_{\beta'}$ phase of phosphatidylcholine bilayers are given by a Landau–de Gennes expression for the free energy containing a term favoring the spontaneous curvature of the membrane. The result is a bilayer that tends to exist with periodic variations in membrane fluidity, i.e., different phases of lipid, separated laterally and in a regular, periodic manner, throughout the bilayer. This phenomenon may have implications for the mechanism of excitability in biological membranes, an idea that is discussed in section II.B.3.e.

A more recent group of experiments from McConnell's laboratory[200-204] involve monomolecular films of phospholipids on water which are studied by epifluorescence microscopy. The monolayers are formed from a single species of phospholipid with a small amount of added cholesterol. The latter species tends to stabilize and lengthen the interface region between different phases (a disordered liquid phase and a crystalline phase) of the bulk phospholipid. Marvelous spiral and branched crystals show up as dark regions in the epifluorescence micrographs (see Gaub et al.[202] (1986) and McConnell et al.[201] (1986)). An interesting property of the spiral structures is that their rotation direction depends on the chirality of the lipid. When a pure optical isomer of a phospholipid is used, the spirals all rotate one way; the other isomer yields spirals of the opposite rotation direction. The investigators find that these patterns are formed reversibly by application of lateral pressure to the monolayer; the reversibility indicates that the structures are equilibrium ones, not nonequilibrium, dissipative structures. However, Möhwald and co-workers have studied similar lipid crystals whose shapes depend on kinetic factors and have fractal properties;[205] these latter examples may, indeed, be nonequilibrium dissipative structures. These phenomena deserve more intensive study as possible examples of self-organization in a system that is biologically important and intimately related to the origin of membrane excitability.

(*c*) *Ionomer Membranes.* Nafion, a perfluorosulfonic acid polymer manufactured by Du Pont, has become a widely studied example of an ionomer membrane with spatially nonuniform structure that displays enhanced transport and selectivity characteristics. The fixed charges in Nafion are negative and, hence, Nafion membranes are cation selective. In 1980, Cutler showed[274] that changes in transport through Nafion were intimately associated with changes in morphology (clustering geometry) of the membrane. More recently, Leddy and Vanderborgh (1987) have shown[275] that mass transport is enhanced when the microstructure of Nafion is made more nonuniform; i.e., the clustering is more pronounced. As described in section III.B.3, numerous theoretical arguments that emphasize the importance of spatial nonuniformity to enhancement of transport in Nafion have also been advanced.

(*d*) *New Methods for Visualizing Patterns.* The experimental study of nonuniformities on a small scale could be advanced if improved techniques for visualizing patterns of ionic concentrations, electric field profiles, etc. were developed. Wightman has developed microelectrodes[276] for the study of concentration distributions in small areas in biological systems, such as near single cells. These microelectrodes can, of course, be used to map out the electric field profile but can also be used to detect concentration distributions if the electrodes are of the ion-selective type. The vibrating-tip electrode developed by Jaffe and Nuccitelli[277] is another tool that has been critical for mapping ionic currents near developing embryos and regenerating structures; it could be applied more broadly to the study of nonuniformities in artificial systems as well. Finally, a variation of scanning tunneling microscopy has recently been developed[278] and appears to map out the electrostatic potential surface near biological molecules; this technique might be useful for increasing the resolution of measurements to near-atomic scale.

3. Theoretical Studies

(*a*) *Self-Organization.* Recent years have seen an increase in the number of theoretical studies of specific biological morphogenesis problems. For instance, Larter and Ortoleva[279,280] proposed a theoretical model for the origin of electrical patterns in the egg of *Fucus*. The model is of the reaction–diffusion type with nonlinear boundary conditions that model the mediation of transport of ions by a membrane-bound species. This transport-mediating species may be a pump or channel but is free to diffuse laterally in the membrane. Being electrically charged, it is also affected by electric fields and tends to move laterally when the rate of electrical migration is greater than that of back-diffusion. Positive feedback results when the ion that is being transported by this mediating species has a charge of the opposite sign to the mediating molecule itself. Interestingly, based on purely theoretical considerations, it was determined that Ca^{2+} was one of only a few possible species that might be involved in this mechanism; the sequestering of calcium ions inside vesicles in the interior of the egg causes its diffusion coefficient in the cell interior to be much less than in the extracellular fluid. This difference in diffusion rates inside and outside the cell is essential in the mechanism by which a symmetry-breaking instability occurs and an electrical pattern results.

Ebeling and Feudel[281] and Malchow[282] also studied the role of charge in reaction–diffusion models by including an electrical migration term in the kinetic equations. Electrical dissipative structures were found in simulations of a Brusselator model that treated the species as ions.

In a recent paper, Fromherz[283] (1988) discusses a scenario by which the charged channel proteins in a fluid mosaic membrane can become self-organized; the mechanism is very similar to that previously proposed by Larter and Ortoleva[279] (1981) but the equations used by Fromherz are more specific to nerve axonal membranes. For instance, the Kelvin cable equation is used to couple the membrane potential to the system, whereas the more general Poisson equation was used by Larter and Ortoleva. Fromherz arrives at much the same conclusions as Larter and Ortoleva for the conditions that can lead to spatial patterns in a membrane system.

Hervagault et al.[130,131,148] (1980) have reported simulations of the kinetics of reactions catalyzed by immobilized enzymes in membranes. The models are of the reaction–diffusion type and show the expected instabilities to temporal and spatial perturbations under appropriate conditions. The simulations described are similar to those reported by Bunow, Kernevez, et al.[284] (1980) for the morphogenesis of the imaginal disc of *Drosophila*. No experimental verification of the expected patterns in an artificial immobilized-enzyme membrane has yet been reported, however.

An interesting pair of papers by Schiffmann[285,286] discusses the effect on reaction rates of patterned distributions of reactants in a membrane. He finds that collision rates are different when membrane-localized species are distributed nonuniformly, and this difference in collision rates would lead to a difference in reaction rates. This effect could have implications for the interpretation of fluorescence data that is routinely used to measure diffusion coefficients of proteins etc. in bilayers. If the fluorescent probe is influenced by this effect, the fluorescence rate that is measured would be different if the membrane is nonuniform in some way. This could lead to a misinterpretation of the data.

The basic equations of transport in a charged system (electrolyte with some type of interface present) are the Nernst–Planck flux equation and Poisson's equation for the electrical potential. In 1979, Rubinstein and Segel showed[182] that this system of equations could become unstable to temporal perturbations and might therefore sustain oscillatory solutions. In 1981, Rubinstein extended this study[183] to consider the role of spatial distribution of ions, particularly the nonuniform distribution associated with concentration polarization near a membrane through which transport is occurring. One important feature of the mechanism by which the oscillatory instability arises involves the nonuniform electric field in the region near the membrane. This is discussed in section II.B.3.d; here we simply reiterate that the mechanisms by which temporal instabilities and spatial instabilities arise cannot be separated and must be considered simultaneously.

(*b*) *Transport in Nonuniform Systems.* In 1981, Versiuijs and Smit[287] derived the local transport equations that would apply in a nonuniform system using the irreversible thermodynamics approach. They pointed out that when the spatial structure of the membrane is taken into account, nonlinearities in the relations between flows and forces will be found; this was originally shown by Kedem and Katchalsky[288,289] in 1963. Versiuijs and Smit showed[287] that the results of this nonlinearity are transport coefficients that depend on the spatial coordinates. The use of spatially dependent transport coefficients is a simple, but nonrigorous way to model spatially nonuniform systems that is often entirely adequate and captures all the important physical features.

Spencer (1984) introduced[290] a simple model for hyperfiltration rejection of electrolytes by charged gel membranes. The possibility of nonuniform membrane structure was taken into account by the introduction of a parameter that described the microhomogeneity of the charge distribution in the membrane. This model appeared to be quite applicable to experimental data in hyperfiltration membranes and could be used for predicting the rejection of electrolytes as a function of concentration and type. However, the model is very empirical in nature and does not lead to theoretical insight into the role of nonuniformity in the rejection process.

Selvey and Reiss[291] (1985) took a more general approach and solved the Nernst–Planck–Poisson equations for a slightly nonuniform system using a perturbation theory approach. Small variations in fixed charge density for a model of a Nafion membrane were used. Their numerical simulation and perturbation theory results showed that an inhomogeneous distribution of fixed charges in the membrane resulted in a higher current efficiency than that calculated for an otherwise identical uniform membrane. The mechanism by which this enhancement arose was interpreted as a difference in forces experienced by anions and cations due to the nonuniform electric potential within the membrane. They also found that the length scale of the nonuniform pattern and its symmetry were important in this enhancement effect.

An important review by Buck[292] discusses the situations in which the Nernst–Planck flux equations are valid. Some investigators have questioned the applicability of these equations in spatially inhomogeneous situations, but Buck finds that the solutions to the Nernst–Planck equations are usually quite good when applied to inhomogeneous materials. However, when the inhomogeneity is on a molecular length scale, as in mosaic membranes, the validity of the Nernst–Planck equations breaks down.

If a membrane possesses a nonuniform distribution of fixed charges, as does Nafion and other ionomer membranes, the possibility exists that the transported ionic species will interact with or even bind to the fixed charge sites. Crank[293] has shown that a fast binding process would result in a diffusion coefficient for the transported species that is dependent on its own concentration. Baird and Frieden[294] (1987) recently presented a rigorous theory for transport in a diffusion cell for a species with a concentration-dependent diffusion coefficient. The equations that were derived could be used to interpret experimental data in order to determine the actual functional dependence of diffusion coefficient on concentration. As Versiuijs and Smit showed[287] for spatially dependent diffusion coefficients, concentration-dependent ones can be used to approximate the effect of the nonlinearity arising from the binding reaction. Buck[292] confirms that this is a valid form of the coefficients in the Nernst–Planck equations and reproduces the effect of binding quite well.

Recent work in our group[295–297] (1986–1988) has shown that enhancement of transport in a spatially nonuniform system can occur when the transport equations are nonlinear. The nonlinearity can result from binding of the transported species to fixed sites (as discussed above) or from other mechanisms, for example, carrier-facilitated transport. Larter and Steinmetz[297] (1988) showed that nonlinearity is always present in transport of ions in a nonperfect conductor since the electrical migration term of the Nernst–Planck equation is nonlinear in the potential. When applied to transport in membranes with nonuniformly distributed fixed charges, this nonlinearity leads to enhanced transport rates. The migration term depends in a nonlinear way on the electrical potential because the concentration distribution of the transported species will vary with the potential distribution. This effect can lead to enhancement of transport by 5–10% over that of an otherwise identical uniform system, a prediction that has been confirmed experimentally.

Many years ago Shlögl[298] emphasized the importance of nonlinear transport behavior in biological membranes or other very thin membranes. Bakshi and Gross[299] showed that electrical conductivity becomes nonlinear whenever the field strength is high, as it always is in biological membranes. Lorenzo et al.[300] used numerical simulations to show that even simple electrode reactions are usually not linear. Hoff[301] showed how to consider nonlinear membrane transport from the point of view of nonequilibrium thermodynamics. And, finally, with a statistical mechanical approach, Eu[302] showed how to take into consideration nonlinearities in transport due to high field strengths.

IV. Opportunities for Development of Applications

Much of the work reviewed here has been carried out in order to arrive at a basic understanding of the origin of temporal oscillations and spatial patterns in membrane systems. Many of the artificial systems have been designed as mimics of biological membranes and are studied in order to achieve a better understanding of the physical and chemical processes that are common to both the artificial and biological systems.

In addition to basic research on these systems, it may be possible, with further research, to develop devices for analysis that utilize these phenomena or to utilize the effects observed here to improve or optimize processes involving membranes. In this section, suggestions for application of these phenomena will be put forward. In some cases, a few preliminary studies bear directly on the possibilities for success of these proposed applications, and these preliminary works will be reviewed.

A. Molecular Recognition

Recently, some groups have investigated membrane oscillators that mimic the biological processes of taste and olfaction. Certain characteristics of the oscillations, such as oscillation pattern, frequency, or amplitude, can be affected by the type of molecule present in the solution bathing the membrane. These membranes, thus, act as molecular recognition devices, and the response observed as changes in oscillation characteristic (pattern, frequency, or amplitude) could be used to "signal" the existence of one of these species in a sample. Practical application of this phenomenon could be made to analysis of biological samples, for instance, after further development work has been accomplished.

The liquid membrane systems studied by Yoshikawa et al. provide one class of molecular recognition devices for alcohols, salts, sugars, amine vapors, and even optical isomers. A liquid membrane formed by placing a nitrobenzene solution containing 2,2′-bipyridine in a U-tube between two aqueous solutions of differing concentration with one containing a surfactant (as shown schematically in Figure 4) produces oscillations whose amplitude is characteristic of the hydrophobicity of alcohols placed in the less concentrated aqueous solution.[143] The amplitude of oscillation is found to increase through the series ethanol → propanol → butanol, which is also the sequence through which hydrophobicity increases. When additional substances, such as NaCl, sucrose, quinine chloride, and HCl were also added to the solution containing the alcohol, the frequency, pattern, amplitude, and even the polarity (up or down from base line) of the oscillation were affected.

By using a chiral surfactant in the less concentrated aqueous salt solution, a detector for optically active glucose can be designed. Yoshikawa et al. found that the frequency of the oscillation in a U-tube device varied with the relative chirality of the surfactant and the glucose but that the amplitude was not affected.[141] However, no discernible pattern in the variation of frequency with chirality was reported, so calibration with a standard would have to be done prior to analysis. A similar system involving a non-optically active surfactant was able to detect the difference between sugars of different molecular weights.[140] Complex oscillations were observed in all cases, but a histogram of the frequencies of oscillation showed a bimodal distribution for sugars with the molecular formulas $C_6H_{14}O_6$ and $C_5H_{10}O_5$ but a single-humped distribution for sugars with the molecular formula $C_6H_{12}O_6$. More studies will need to be carried out before this class of molecular recognition devices could be applied to an actual analysis.

A second group of studies on molecular recognition oscillators involves DOPH-doped Millipore filters placed between aqueous KCl solutions of different concentrations. The addition of various foreign substances to the less concentrated side has a dramatic effect on the frequency of oscillation in many cases.[118] The substances NaCl, H^+, and additional KCl all increase the frequency, whereas the addition of sucrose to the less concentrated side decreases the frequency. An interesting case is that of $CaCl_2$, which shows little effect on the frequency up to a concentration of about 20 μM, above which oscillation ceases. This is to be contrasted with the other substances studied whose concentrations can be varied over several orders of magnitude before oscillations will cease; the authors suggest that this phenomenon may somehow be related to the special role that Ca^{2+} plays in biological membrane processes.

Both of the above sets of experiments might be thought of as modeling the taste process; a final experiment from Yoshikawa et al.[303] is suggested to model the biological process of olfaction. In this system, a membrane of oleic acid with propanol and tetraphenylphosphonium chloride is placed between aqueous solutions of NaCl and KCl of identical concentrations. The arrangement is different from the U-tube experiments in that the organic layer is floated on top of the two aqueous solutions which are, in turn, placed in separate compartments beneath the organic layer. Amine vapors dissolve into the organic layer from above and can be detected by changes in the frequency and shape of the electrical oscillation; the changes are characteristic of the chemical identity of the vapor. The types of vapors tested include NH_3, methylamine, pyridine, and piperidine. Whether or not this system accurately models the olfaction process, it may be another possible molecular recognition device, this time useful for the analysis of vaporous species.

B. Quantitative Analysis

An application closely related to that of molecular recognition involves the detection and measurement of concentration of molecules placed into the bathing medium of a membrane oscillator. Meares and Page published[63] an excellent investigation of the effect on the frequency of the Teorell oscillator of various properties of the membrane but did not fully exploit the dependence of the frequency on the concentration of the salt solution which carries the current through the membrane. The theory that they developed provides a formula for the frequency which depends on membrane characteristics such as surface charge density, pore density, and hydrodynamic permeability as well as on the concentration of the solution on either side. The calculations reported all agree very well with experimental observations, but only the membrane characteristic parameters were varied in their study. The formula could then be used for quantitative analysis of salt. Further development work and extension of this approach would be needed before a working quantitative analysis device could be designed.

Several years after Meares and Page's study, Hervagault et al.[130,131] reported oscillations in potential across an immobilized-enzyme membrane. The membrane contained acetylcholinesterase immobilized in albumin which was subsequently cross-linked with glutardialdehyde. The membrane is placed between two flow compartments, through which are pumped phosphate buffer; acetylcholine, the substrate for the enzyme, is injected in one compartment and oscillations are observed. The frequency of the oscillation can be seen to vary with the concentration of the substrate in their published results, but no mention of this is made. This system could provide an interesting enhancement of selective-electrode technology which takes advantage of nonlinear dynamics. Species-selective electrodes currently exist that utilize a membrane containing an immobilized enzyme. A substrate for the enzyme diffuses through the membrane, reacts at the enzyme site, and releases a product that is usually detected as an ion (generally H^+) and an associated voltage signal picked up by electronics attached to the membrane electrode. The possibility of an oscillatory reaction with a concentration-dependent frequency adds an additional degree of freedom that has not yet been exploited in the selective-electrode field.

A detailed study of the dependence of a membrane oscillator's frequency on concentration of a substrate was not reported until 1984. Yoshikawa and Matsubara showed[138] that the frequency of oscillation in the liquid membrane formed from picric acid in nitrobenzene was sensitive to the concentration of alcohol in one of the aqueous solutions. The frequency was found to vary with concentration of the alcohol according to an equation reminescent of the Langmuir adsorption isotherm:

$$\frac{C}{\log f} = \frac{1}{\log f_{\max}}\left[C + \frac{1}{K}\right]$$

Here, C is the concentration of alcohol, f is the frequency of oscillation, $f_{\max}$ is the maximum frequency, and K is a constant. A similar relationship was found many years ago in a study of the response of nerves to a taste stimulus. An explanation provided by the investigators is that the alcohol is incorporated into the surfactant monolayer at the interface and the alcohol's presence affects the formation of micelles, which is critical in the proposed mechanism of oscillation. As in the Meares and Page approach, this equation could be used to allow a quantitative determination of concentration (now of an alcohol, rather than a salt) from an oscillation frequency if the necessary calibration were done ahead of time.

C. Enhancement of Transport

Experimental studies of ionomeric ion-selective membranes such as Nafion indicate that their selectivity is much higher than might otherwise be expected on the basis of the total number of fixed charge centers in the membrane. Experiments of Cutler[274] and Leddy and Vanderborgh[275] indicate that this enhanced selectivity is due to an enhancement of transport by the microheterogeneous, or clustered, structure of these materials. Larter and co-workers have shown[295-297] that such an effect would be expected if the transport laws in these systems were nonlinear, a situation that could hold for simple electrical migration of the Nernst–Planck type through a fixed-charge region. Selvey and Reiss[291] and Steinmetz and Larter[297] have published numerical studies showing that transport via the Nernst–Planck equation is enhanced in a system with nonuniform electrical potential such as might be expected to exist in these systems.

All of these observations indicate that nonuniformity might be a useful means to enhance transport in systems where it is desired to optimize the transport rate, such as membrane separations. An example is provided by the translational motion of chromosomes which occur in nonuniform electric fields.[304] Theoretical studies to date have revealed very few restrictions on the types of nonuniformity (i.e., pattern type, characteristic length scale of pattern, etc.) that lead to enhancement. Further research on specific systems is needed to determine whether any restrictions exist. Another open question is whether the nonuniformity need be of an orderly type or random. If the nonuniformity is the result of a pattern-formation mechanism (such as a reaction–diffusion instability) or some type of self-association as may exist in the formation of ionomers, the nonuniformity would be associated with some degree of order. This is the type of nonuniformity that has been studied theoretically, but it is still an open question as to whether order is a necessary condition for the existence of this effect or whether random nonuniformities will lead to enhancement of transport as well.

An extension of these ideas to include systems in which chemical reactions are also occurring might be advantageous. Borchert and Buchholz (1984) have argued[305] that a nonuniform distribution of enzyme catalyst immobilized in supporting beads leads to greater efficiency when the conditions are mass transfer limited. This effect is simply due to the enzyme being placed closer to the surface of the bead in their experiments rather than spread evenly throughout the bead, eliminating the time required for substrate to diffuse into the supporting matrix to reach the catalyst. More complex behavior could occur in such a system, though, if the chemical kinetic and transport processes were occurring on the same time scale rather than being limited by the rate of diffusion. Also, if the reaction were nonlinear in some way, as is often the case with enzymes, further enhancements of enzyme effectiveness might be found. This idea is quite speculative and should be investigated further and in more depth.

V. Implications for Biological Phenomena

A. Pacemaker and Neuron Behavior

We have reviewed here many studies of biological and artificial systems that are relevant to the phenomena of nerve conduction and interaction of nerve impulses in synaptically connected neurons. The mechanism by which the action potential arises is still not fully understood. It seems clear that excitability is intimately related to ion-transport processes that occur in the membrane, but it is not at all clear how or whether the *details* of these processes have any effect on the observed macroscopic phenomenon.

In the electrophysiological community, excitability is widely studied in terms of specific ion channels or conductances, and the overall progress in the field moves toward finding out more and more detail regarding the molecular structure of these channels and the molecular events (conformational changes etc.) that occur during the opening and closing of the channels. Some, but not as many, investigators have been concerned with the role of the lipid bilayer in ion transport; this role is often thought of as a "passive" one, though, involving the leakage of ions through the bilayer, usually under the influence of a great concentration gradient. The role of the bilayer itself in the excitability phenomena has not been widely studied by this group of investigators.

The studies reported here of artificial membrane systems that display excitability but with no ion channel proteins present must be addressed in the overall scenario of biological nerve action. The early work of Pant and Rosenberg[35] could be criticized as not really relevant to the biological system since the lipid bilayer was used to separate two compartments that were very far from physiological conditions [KI(aq) at pH 10 and $K_3Fe(CN)_6$ at pH 5]. This criticism must now be put aside; as described above, an important experiment by Antonov et al.[109] in 1980 showed excitable behavior in a bilayer membrane separating identical buffered solutions at physiological conditions. Similarly, several recent experiments by Yoshikawa et al.[127,129] have demonstrated the existence of excitable behavior for a variety of bilayer membranes, some placed between KCl and NaCl solutions at physiological conditions, a situation closely mimicking the inside and outside of a typical neuron. These studies indicate a link between a localized phase transition of the bilayer and the excitability property since no ion channel proteins are present in the bilayer.

Another result of the work described in the above sections is the clear relationship that exists between excitability and the ability to sustain oscillatory behavior. The action potential is simply a single pulse of an oscillation that could become a sustained oscillation at slightly different conditions or with simulation by, for example, synaptic connection to another electrical source. Indeed, spontaneously oscillatory pacemaker cells (neurons or secretory cells that produce spontaneous electrical pulses) abound in biological systems and are not really different from the ordinary nerve; it is simply the surrounding conditions, or environment, that are different. Any studies that help to extend our knowledge of membrane oscillations will, therefore, have some bearing on our understanding of the origin of the action potential.

B. Pattern Formation in Growth and Development

Reaction–diffusion theory for biological development and growth processes has still not been enthusiastically embraced by the biological community. Harrison[306] in a recent review sees the reluctance to accept the approach espoused by Turing and subsequent investigators as primarily philosophical. He sees the problem as a lack of a consensus on a preconceived idea aboput what leads to form in biology. Three ideas regarding the origin of form in biology are represented in the biological community: the structural approach sees form as arising by simply the fitting together of static shapes to make a whole; the equilibrium approach envisions form as arising from the minimization of free energy, as in the appearance of form in soap bubbles; and the kinetic approach, of which reaction–diffusion theory is the most viable, sees form as arising primarily by movement away from equilibrium via chemical reaction or transport process. From a physical chemistry point of view, the latter approach is the most valid since (1) living systems are certainly not close to thermodynamic equilibrium and (2) the fitting together of static shapes to produce a more complex form begs the question of the origin of the shapes and the mechanism by which they are put together in that particular manner.

Another problem that impedes the acceptance of reaction–diffusion theory as an explanation for biological pattern and form is confusion surrounding the notion of "morphogen" as originally proposed by Turing. In Turing's hypothesis, a morphogen is a molecule whose distribution becomes nonuniform over a uniform field of cells by the action of chemical reaction and transport processes and which, subsequently, directs or signals the cells in the field to develop at different rates according to the concentration of the morphogen. In recent years, "morphogen" has also been used to describe the substance which diffuses along some sort of preexisting gradient, signaling the cells to develop in a certain way, but which may not itself be involved in the process by which the gradient arises. This definition of morphogen is unfortunate since it also begs the question of the origin of the preexisting gradient and does not, therefore, lead to a satisfying and self-contained theory of the development process.

Some recently discovered "morphogens" that may or may not satisfy Turing's more rigorous definition include auxin[306] (indoleacetic acid), implicated in the generation of vein patterns in leaves; an undecapeptide[306] in *Hydra* associated with the development of tentacles; calcion ion,[272] implicated in the development of *Acetabularia* as well as in many other developmental systems; and retinoic acid,[307] a molecule recently claimed to be the first true morphogen discovered, found in chick limb buds. Of all of these, calcium ion is the most likely link between tissue- or organ-level development processes and the cell membrane of interest in this review. In Berridge and Rapp's 1979 review[5] of biological oscillators, calcium ion was singled out as the species most likely to couple the membrane-localized and cytoplasm-localized oscillations. Not only is calcium involved in the control of transport of other ions through the cell membrane, it is also heavily sequestered in the cell interior, ready to be released rapidly upon the reception of some sort of signal, usually a pulse of calcium influx. Because of these special properties, calcium is an important species connecting the growth and development processes to the membrane excitability phenomena reviewed here.

Another connection of interest in this review is that which exists between oscillatory events and spatial patterning in developing systems. This connection has been well studied in the slime mold *Dyctiostelium discoidium*, and analogies have been drawn between this system and the Belousov–Zhabotinskii reaction[308] in which similar patterns develop, also by spatial and temporal organization of waves emanating from localized oscillators. It may be that more such connections will be discovered, particularly involving membrane oscillations and electrical potential patterns. The oscillations[8–14] and spatial patterns[271] discovered near developing bean roots are a good example that has not been studied in depth. Other systems including *Acetabularia*[236] and *Pelvetia*[238] have been shown to have such a connection.

C. The Nature of Intelligence

A more speculative avenue for application of the results reviewed in this article is the area of the nature of consciousness and intelligence. We are certainly a long way from being able to draw hard conclusions about these phenomena, but research into the behavior of neural networks, both real and artificial, is laying the groundwork for an eventual attack on the problem. The fact that individual neurons can be spontaneously oscillatory and that synaptic coupling of neurons can generate chaos leads to the question of whether chaos is the normal operating mode of a network of neurons or whether it is pathological or a sign of disease. Research is already underway from both the physiological point of view[309] and the psychological one.[310] In the former study,[309] evidence of chaos in mammalian EEG signals is presented; in the latter,[310] a theory of the thought process utilizing the ideas of chaos is presented. The research reviewed in this article is of the type that enhances our understanding of the dynamical behavior of *individual* neurons and will have an impact on our understanding of *systems* of neurons such as the brain itself.

VI. Conclusions

We have reviewed here the phenomenon of spontaneous oscillation in membranes and the close relationship between this phenomenon and that of spatial patterning or nonuniformity in the membrane. It has been shown that spontaneous periodic oscillations are but one manifestation of a propensity to excitability, a property that is possessed by neurons and pacemaker centers in biological systems as well as by artificial membrane systems, originally designed as models of the biological ones. In addition to providing insights into the physicochemical origin of this behavior in biological systems, the artificial systems are inherently interesting nonlinear dynamical systems in their own right. Possible uses of these systems as analysis devices constitute a relatively unexplored application of these phenomena. It is hoped that more basic research on these systems will continue to provide further insights into the biological phenomena of excitability, growth and development, and possibly even, eventually, a better understanding of the nature of consciousness and intelligence.

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