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On the Role of the Phospholipid Bilayer Membrane in Free Energy Coupling

by

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Abstract.

The lipid bilayer response to local perturbations is determined from thermodynamic monolayer phase diagrams. Entropy, forces, and fluctuations in the phospholipid monolayers quantitatively describe chemiosmotic coupling, propagating action potentials, and ion channel opening and closing in bilayer membranes. Falsifiable predictions are derived and compared with experimental observations in pure and biological lipid bilayers. Paradoxes in previous one-dimensional membrane theories are resolved. The role of the phospholipid bilayer is therefore to provide the physical mechanism of biological membrane function.

I. Newton, Principia. Rule I: We are to admit no more causes of natural things than such as are both true and sufficient to explain their appearance.

INTRODUCTION

I. Dimensionality

Membrane theory has traditionally been based on thermodynamics (it is worth recalling References 1-44), but virtually every previous theory was confined to one spatial dimension only, that of the direction across the membrane from the exterior to the interior compartment of biological cells and organelles. In this direction, the membrane is microscopic, constituted by a bimolecular layer of lipid molecules into which the membrane proteins are embedded.

The membrane functions we shall be describing are, however, macroscopic: Free energy coupling between chemical reactions and osmosis (29;30;37;40; 44) is of the order of magnitude of 1 kcal/mole catalysis. Action potentials propagating along axons (22;45;46;47;48; 27;49) extend for 10^{-3} sec in time and for 10^{-3} m in space, approximately. These clearly are macroscopic with respect to microscopic collision times below 10^{-8} sec and microscopic lattice constants in the liquid-crystalline

membrane fluid of the order of 10^{-9}m. This is the reason why these previous, microscopic theories failed to provide the physical mechanism of biological membrane function.

Biological membranes are macroscopic in the two dimensions along the membrane plane which are established by the bimolecular lipid layer. We shall apply thermodynamics to the three lipid bilayer dimensions for the first time. There have been previous attempts to describe two-dimensional aspects of membrane function, such as Lorente de No's or Tasaki's approach to the action potential or Kell's approach to chemiosmosis, but the mechanism of the described functions was not identified.

II. Thermodynamic response

I describe in this review the normal thermodynamic response of the lipid bilayer to any perturbation (reviewing Refs. 50-57). One such perturbation will be the effect of local protein activities to the phospholipid microenvironment (58). Only the lipid bilayer will be described, and the proteins will be represented by the perturbation of the lipid variables they cause.

The most important result that we shall obtain is a resolution of the Maxwellian paradoxon. It will turn out that the Maxwellian "Demon" violates the Second Law only because thermodynamics is applied to the wrong system: the one-dimensional microscopic membrane model. By consideration of the other two dimensions as well, the Maxwellian "Demon" is actually the c o n s e q u e n c e of

classical thermodynamics.This solution is consistent with an earlier prediction (59).

The theory provides a physical mechanism which unifies local and global chemiosmotic coupling with the propagating action potentials as well as with the fluctuating membrane ion channels observed in biological and pure lipid bilayer membranes. It establishes the link between physical liquid crystal theory (60;61;56) and biological membrane theory. It resolves paradoxes in microscopic interpretations of membrane functions (cf. 1;62;63;45;64;31; 36; 34;27;49;50;65;53;66). Nevertheless, it is compatible with the notion of "transport protein" complexes (46;67;27) supposed the complex includes the perturbed lipid bilayer. The mechanism of membrane transport is identified entirely in the lipid component of the membrane.

The lipid mechanism thus plays a similar role in understanding membrane function as acoustics played in the theory of Musics (68). It is though more sophisticated in the case of the membranes due to the two-dimensionality which creates the possibility of new types of thermodynamic properties with respect to the third, trans-membrane direction.

III. Monolayer diagrams

A local perturbation in the macroscopic two-dimensional lipid bilayer induces the normal thermodynamic response (54; Fig.1). The generalised thermodynamic potential (69;70) is increased above its equilibrium value, and

thermodynamic forces appear which drive dissipative or reversible transport. The thermodynamic fluctuations are also increased or decreased by the perturbation.

The lipid response can be predicted quantitatively from the thermodynamic phase diagrams (54), i.e., from the dependence of the forces on the extensive variables, since the phase diagrams are measurable independently. Such phase diagrams are obtained from calorimetry (71;72;73), from two-dimensional surface pressure/area monolayer diagrams (Fig.2 from Ref.39; see also 74-82), and from chemical titrations measuring the dependence of particle numbers on the electrochemical potentials (83;84;85). In general, phase diagrams also involve measurement of the cross-terms relating those (idealised) intensive forces (temperature T, pressure P, surface pressure $\tilde{\pi}$, electrochemical ion potentials μ_i) and extensive variables (energy U, volume V, surface area A, particle numbers N_i) which are not thermodynamically conjugated in the real system.

IV. Assumptions

In this way, I arrive at quantitative predictions from the phase diagrams. No adjustable parameters are introduced. It is easy to falsify this theory, and experimental verification is therefore meaningful. Three assumption are made in the following:

1. The validity of classical thermodynamics. This is not trivial. The local perturbation will be described by local potentials only defined in equilibrium. This assumption is

sometimes called "local equilibrium" (19;34). In principle, local perturbation requires a formulation of the theory in terms of functionals (69) which we have not derived. The presence of the generalised thermodynamic potential is clearly established in equilibrium thermodynamics. Assuming the continuity of physical theory, I shall assume its existence in nonequilibrium and the validity of the local equilibrium approximation.

2. The linearity of the perturbation. In most of the quantitative predictions, the responses will be linearised with respect to the perturbation. We shall, however, apply nonlinear symmetry analysis (60;61) in the case of the all-or-none action potential.

3. Thermodynamic lipid monolayer states in biological bilayer membranes are assumed to be equivalent to monolayer states observed at an air-water interface. The quantitative predictions made from monolayer phase diagrams have therefore to be taken c u m g r a n o s a l i s . They represent estimates of order of magnitude only. The existence of order transitions, continuous or discontinuous, in both monolayers and bilayers, is crucial for the theory. This assumption, however, can no more be disputed (84;39).

Fig.2 is one such phase diagram. It will be used for the quantitative estimates made in the following. But phase diagrams with respect to further variables and other lipid

compositions are required in general. The method to predict the lipid responses will always be the same. The lipid in Fig.2 has been chosen because this lipid had been studied in great detail in the past. It represents a general phospholipid model due to the methylation of the second protonable negative phosphate charge (86;87). It exhibits the typical Van der Waals behavior of many two-dimensional and three-dimensional real gases and fluids. In this case, the two-dimensional Langmuir phase diagram of the dependence of surface pressure on area per lipid molecule is shown, at different values of the electrochemical proton potential.

V. Predictions

Fig.3 demonstrates the method how to obtain quantitative predictions from such phase diagrams. The corresponding equations are presented in the following sections. Quantitatively, we thus obtain (55)

1. free energy coupling (54), i.e. the work performed during a perturbation of the lipid state;

2. the forces produced and the consequent propagation velocity (56;54);

3. the strength of the area fluctuations (54;57).

Usually, free energy coupling is weak, ca. 0.1 kcal/mole protonation in Fig.2, and the relative area fluctuations are small, ca. 1% as follows below in Sections A and C. The monolayers therefore usually represent a non-

fluctuating barrier with no significant function in membrane coupling. The assumption of an inert, non-fluctuating lipid barrier (i.e. vertical slope in Figs. 2,3) is fundamental in the molecular models of membrane function. Only if this assumption is made, can the membrane functions be attributed to isolated proteins. Nevertheless, this assumption is approximately confirmed by our theory for the more incompressible states of the lipid monolayers.

Propagation velocities would be infinite for incompressible states. They are as large as 20 m/sec maximally in the more crystalline states at high surface pressure seen in Fig.2. Isentropic phase diagrams have however to be used for the determination of adiabatic propagation velocities, in contrast to the isothermal phase diagrams required for prediction of the equilibrium fluctuation strength.

As can be seen in Figs. 2,3, the assumption of an inert lipid barrier breaks down in "critical regimes" of thermodynamic lipid states where the slopes in the phase diagrams become rather horizontal. In these regimes, the susceptibility of the lipid monolayers to external perturbation is dramatically increased. Following Sections A, B, and C, in the center of Fig.2 above 10% relative area/thickness fluctuations and above 1 kcal/mole protonic free energy coupling can be seen. Such values appear for the integral work performed on the phospholipid state. For

adiabatic, reversible coupling, the efficiency of free energy coupling is 1. Interestingly, as indicated in Fig.3, the propagation velocity slows down in such critical regimes, and would become 0 m/sec at a critical point.

Critical points (88;89) with disappearance of propagation and divergence of the fluctuations have not yet been established in biological membranes. The response theory is however not limited to critical points. At any critical regime, whether continuous, first order, or of critical, second order (compare top, center, and lower part of Fig.2), the lipid bilayer represents a compressible, fluctuating thin layer and not an inert barrier.

VI. Unification of membrane theory

Fig.4 represents the three aspects of the thermodynamic response of the lipid bilayer to local perturbation. The negative phospholipid surface charges are omitted. Perturbation by catalytic protons (+) is taken as example.

1. The local perturbation performs work on the lipid conformation. Coupling is reversible in the absence of hysteresis in the phase diagrams. This reversible work, or free energy, can be expressed depending on the type of phase diagram used, either in terms of chemomechanics similar to that developed for one-dimensional polymers (90;34), or in terms of chemiosmosis (29;30;40) similar to that developed by Mitchell, Williams, and Kell for global or local coupling.

2. Propagating responses arise which are either reversible or dissipative. In this way, free energy is propagated, or delocalised, along the phospholipid surfaces - and also across the lipid bilayer in the presence of hydrophilic defects (53). Such membrane excitation couples all thermodynamic variables, if allowed by symmetry principles, and can therefore be detected as a simultaneous excitation in temperature, surface pressure, and the electrical or chemical membrane potentials.

3. The thermal motion in the lipid bilayer results in the formation of hydrophilic defects across the hydrophobic bilayer lattice. The statistical defect probability is significantly increased, by order of magnitude, in the critical regimes of the lipid states. This aspect of the thermodynamic lipid response opens the possibility of formation of ion channels and trans-membrane coupling.

This article proceeds as follows. In section A the fluctuations will be treated quantitatively and falsifiable predictions will be derived. The threshold for channel induction, the reversiblity of opening and closing, and the statistical lifetime and discrete amplitude distribution are predicted. As a special case, voltage-clamp and the resulting increase in membrane permeability are predicted.

In section B voltage-induced action potentials are described. The classical experiments of Cole and Curtis (64) which originated the Ohmic interpretation are

reinvestigated; it is proven that, within experimental accuracy, the observed membrane current is completely reversible and the true Ohmic current during the action potential is zero. A detailed mechanism for the formation and the propagation of action potentials is derived, following Ref.(56) using methods developed in the hydrodynamic theory of smectic liquid crystals C* (61). Time reversal symmetry of the observed electromechanical coupling directly demonstrates, again, that the action potential is a reversible, isentropic excitation free of Ohmic currents. Parity symmetry is used to demonstrate from the observed coupling that macroscopic chirality is required for the nerve action potential. The propagating density pulse is in this way coupled to the ordered rotation of the electrical dipoles. This rotation is measured as the reversible electrical membrane current and depolarisation. It is argued that only the lipid bilayer provides macroscopic chirality in biological membranes and that any mechanism located in single protein molecules would require that physical laws change with the arbitrary choice of the direction of the spatial coordinate system.

In section C free energy coupling and transport along and across lipid bilayers is considered. The interplay in the two-dimensional lipid bilayer between entropy, forces, and fluctuations will provide a physical mechanism of chemiosmosis. The controversy between localised and global chemiosmotic coupling is resolved. The conditional opening

of the lipid bilayer by the fluctuations finally resolves the contradiction between the Second Law of thermodynamics and the Maxwellian "Demon."

Each of the lipid responses predicted has in part been experimentally investigated. The results will be cited. The presence in synthetic lipid bilayer membranes of ion channel fluctuations, of millisecond action potentials, and of conformational, free energy coupling transitions during protonation establish the validity of the theory.*

Specific protein activities such as acetylcholinesterase (AChase) and sodium-activated adenosintriphosphatase (Na-ATPase) are used as specific perturbations. ACh-induced and ATP-induced ion transport are predicted with the correct direction and ion-specificity. It is therefore concluded that the lipid mechanism is thermodynamically evident and quantitatively sufficient for the described specific functions of biological membranes.

* The new feature of our solution is the explicit consideration of the membrane bilayer as a thermodynamic system. The entropy S of the aqueous lipid monolayer surfaces unifies free energy coupling, forces driving propagating excitations, and fluctuations. This is mathematically expressed in the Taylor expansion $S = S_0 + \sum_i \frac{\partial S}{\partial n_i} \delta n_i + \sum_i \sum_j \frac{1}{2} \frac{\partial^2 S}{\partial n_i \partial n_j} \delta n_i \delta n_j + \cdots$

The perturbation gives simultaneously rise to the coupled entropy S_0, the forces $\frac{\partial S}{\partial n_i}$, and to a well-defined variation in the strength of fluctuations, measured by $\frac{\partial^2 S}{\partial n_i \partial n_j}$.

A. FLUCTUATIONS

The lipid bilayer is as every macroscopic system subject to the thermodynamic fluctuations. In contrast to other, three-dimensional systems, the microscopic thickness of the bimolecular layer implies that the thermal motion dramatically alters the properties of the system with respect to the third dimension. Macroscopic flows in this third dimension across the bilayer between the two aqueous compartments on either side of the membrane appear even if only microscopic, but hydrophilic defects would arise in the bilayer due to the fluctuations. Therefore, bilayer membranes represent a unique thermodynamic system where the microscopic defect fluctuations can be directly observed.

VII. Defect fluctuations

The presence of fluctuations, whether in pure synthetic or in biological bilayer membranes, is thermodynamically evident. It follows from thermodynamic principles that any defect which is not forbidden will also occur in equilibrium with a certain probability. The flexibility of the polymeric phospholipid hydrocarbon chains does not provide any way to avoid the formation of hydrophilic defects. The microscopic activation energy had to be known to make quantitative predictions about the probability of each defect which is possible. Anyway, fluctuating and hydrophilic defects will appear by necessity in equilibrium.

--- Strength of area/thickness fluctuations

The lipid phase diagrams, e.g. Fig.2, quantitatively predict the increase of defect probability by at least one order of magnitude in the critical regime of lipid states. The induction of hydrophilic, ion-conductive channels across the bilayer with increased probability above a threshold of the applied perturbation is therefore predicted.

The strength of the thermodynamic fluctuations has actually been quantitated for the first time by Einstein (91). The Boltzmann principle already states that in equilibrium the probability of each allowed state increases exponentially with its entropy (2). The equilibrium state is the most probable state. With reduced, but finite probability, neighbouring states also appear. The probability of states remote from the most probable state (e.g., remote from an impermeable lipid bilayer state) therefore increases with the curvature radius of the entropy function at its maximum (Fig.1). This curvature is proportional to the derivative of the extensive variable with respect to the force under consideration. This derivative is the corresponding susceptibility of the system, i.e., the strength of the response of the extensive variable to the perturbation by the forces.

Fig.1 demonstrates the increase of fluctuation strength with entropy curvature radius. Fig.2 predicts increase of the area fluctuations by more than one order of magnitude when the electrical or the chemical proton potential is increased toward the apparent "pK". The exact result of thermodynamic fluctuation theory is for variable area A and conjugated force π/T (88;92;57):

$$\langle (\delta A)^2 \rangle = -kT \left(\frac{\partial A}{\partial \pi} \right)_{T, \mu_i}$$

The strength $\langle (\delta A)^2 \rangle$ of the area fluctuations, $\langle\ \rangle$ being the statistical mean, is in equilibrium proportional to the isothermal two-dimensional compressibility of the lipid monolayers. The values obtained from Fig.2 are: 1% relative area fluctuations at steep slope, and above 10% in the critical regime in the center of the Figure.

IX. Predictions for ion channels

We arrive at the following falsifiable predictions from Fig.2. These are with quantitative modifications valid for general Van der Waals-type phase diagrams of any phospholipid (74;93;94):

1. The lipid bilayer membrane represents usually a rather stable dielectric barrier which prevents the passage of ions, because the strength of the area/thickness fluctuations is small in most lipid states, whether in the more liquid or in the more crystalline regime.

2. However, in every state, there is a certain probability for the formation of defects. These include hydrophilic defects which dramatically alter the observable trans-membrane permeability. The finite probability gives rise to the presence of defects in every state. This implies a resting membrane permeability in the absence of perturbation. Usually, this "resting resistance" is high. It may however dramatically depend on the resting lipid bilayer state.

3. The reversibility of phase diagrams such as Fig.2 predicts that defect formation is reversible. Any individually observable hydrophilic channel across the lipid bilayers must open and close reversibly in thermodynamic equilibrium, for in principle infinite times.

4. The reversible thermal motion will not irreversibly alter the bilayer lattice. Any reversibly fluctuating ion channel will therefore be surrounded by a stable and impermeable two-dimensional lattice of hydrophobic hydrocarbon chains. Fig.5 gives an electrode-view of the defectous two-dimensional hydrophobic lattice. Each point represents one hydrocarbon chain. Lattice constant is in average ca. 4.5 Å (95). The microscopic channel structure and hydrophilic channel boundary cannot be described by the macroscopic theory. The flexibility of the hydrocarbon chains allows nevertheless the formation of a topologically continuous surface across the channels constituted of hydrophilic lipid phosphate head groups.

Like a "Möbius Band" (96), the bilayer now has only one single surface (Fig.5,6).

The appearance of aqueous defects is predicted, both in the resting and in the strongly fluctuating critical regimes. Discrete "pore" size and conductivity due to the lipid chain lattice is expected. In this sense, our theory is compatible with the previous interpretation of the observed

ion channels by pores (97-110;41; see also Refs. 50;111-114).

5. The lifetime of channel-opening fluctuates due to the thermal motion. No deterministic defects of fixed open time will appear in equilibrium. The mean lifetime is a property of the lipid bilayer, no matter whether the fluctuations are induced by proteins or by other perturbations of the thermodynamic lipid variables.

6. The induction of ion channels is characterised by a threshold (Fig.7) defined by the value of the required isothermal compressibility. This value is however not yet known. Still, the compressiblity dramatically increases in a critical regime of states. This critical regime can be induced from any initial state by appropriately altering any of the lipid variables. The induction of ion channel fluctuations is predicted: by the electrostatic potential ψ, by pH, by pCa, and by other chemical potentials. Any ion channel fluctuation depends, e.g., on lipid monolayer surface pressure and temperature. Quantitatively, the threshold value depends on the initial state. It is large when this resting state is remote from a critical regime. The voltage or pH threshold for instance is larger when the apparent phospholipid pK is more acid. In the low surface pressure regime (Fig.2, bottom right), the monolayer is also strongly fluctuating. In contrast to

the critical regime, it is however not stabilised by the nonlinear regime and not limited by less fluctuating lipid states. The bilayer is less stable here and presumably breaks; such states will not be further considered.

The characteristic unity of the threshold perturbation, required to reach the critical regime, can be read from Fig.2: for voltage-induction the electrochemical unit at room temperature, 60 mV; 1 pH unit for acidification from neutrality; 1 dyne/cm for induction by a change in surface pressure.

7. Desensitisation is predicted for too strong perturbations which complete the order transition into a less compressible phase. E.g., below the apparent phospholipid pK (Fig.2, left), or above the critical regime of surface pressure (Fig.2, top), the increased fluctuations must disappear again.

Observations

Each of these predictions has been experimentally tested. Biological membranes (115;101;116) and also pure lipid bilayers (Refs. 117-123; 66; 55) are in general

1. stable dielectric barriers

2. with background conductivities in the order of magnitude 1 to 1000 pS.

3. This is the magnitude of resting conductivities as well

as of the discrete ion channel conductivities.

4. Resolved ion channels open and close reversibly.

5. The statistical lifetime is of same magnitude, 1 to 100 msec, in biological, reconstituted protein-lipid, and in pure lipid bilayer membranes (compare, e.g., 116; 109;with 66).

6. Biological ion channels depend as predicted on the phospholipid variables. The dependence on voltage (124;125)and on calcium, pH, pressure and temperature of protein-induced ion channels is in many cases an established fact (126;109). Pure lipid bilayers have similar threshold values for ion channel induction. Examples are the voltage-induced (127;57) and pH-induced ion channels (66). Threshold values are between 50 to 200 mV or 1 to 4 pH units below neutrality.

7. In the case of pH-induced ion channels, the reversible desensitisation at very acid pH below the apparent pK has also been reported (66).

Apart from the lack of quantitation in the surface pressure-induced lipid ion channels, and the only preliminary results with respect to calcium-induced ion channels in synthetic lipid bilayers, the predictions of the theory have clearly been confirmed within experimental accuracy. No other ion channel theory has been capable to explain the observations. Therefore, it is by now to be

considered an established fact that ion channels open and close due to the reversible motion in the lipid bilayer.

XI. Control by proteins

The thermal motion in the lipid bilayer is also controlled by local protein activities which alter the local lipid isothermal compressibility. In this way, proteins allow for specific control of ion channel fluctuations. The specificity of ion channel induction is increased by choosing initial lipid resting states remote from critical regimes. In this case, strong perturbations only suffice to induce the threshold and only specific proteins will be capable to open the ion channels in the lipid bilayer. Therefore, the lipid fluctuations are completely compatible with the observation of the control of ion channel opening and closing by proteins.

In contrast to molecular ion channel models using amino acid sequences of polypeptides and proteins, our theory provides a physical mechanism. This mechanism is thermodynamically evident and directly confirmed by crucial experimental observations. The molecular ion channel model must therefore be considered an intestable hypothesis. It is incompatible with the observation of the presence of ion channels in the pure lipid bilayer. Moreover, microscopic time scales are too fast, and spatial dimensions are too small, to explain the observed channel open times and conductivities.

A detailed application of the lipid mechanism to the

acetylcholine-induced ion channels has been described earlier (50;51; 66;54).

XII. Voltage-clamp

In the next Section, I proceed to the description of the action potential and the related propagation phenomena. We shall not come back to the equilibrium phenomena described above and therefore keep in mind:

Voltage-clamp, i.e., the relaxation of the membrane to another macroscopic equilibrium state, alters the strength of the ion channel fluctuations. Above some threshold voltage, defined by the critical regime, the permeability of the bilayer membrane is crucially increased due to the increased strength of the thermal motion. This result is in agreement with the observation by Hodgkin and Huxley that the Ohmic, irreversible membrane current is increased (22;47) and the passage of ions (128) is observed during voltage-clamp. The result is also in agreement with the voltage-induced ion channel fluctuations observed in vivo (124) The clearest experiments on voltage-clamp on pure synthetic phospholipid bilayers have been performed (57) using the generally successful method of patch-clamp glass pipettes.

The kinetic S-shaped "on" and exponential "off" response to voltage-clamp lead to the m^3h and n^4 description of the Ohmic currents (49). It is expected from the slowing-down (S-shape) on the more fluid ("on") side of the transition (Figs. 2,3,-). Whatever the detailed kinetics and its

dependence on sodium and potassium, non-propagative voltage-clamp experiments demonstrate clearly that the induced membrane currents are irreversible (Fig.7) and hence of Ohmic nature.

A completely different result will now be obtained for the propagating action potential.

E. FORCES

E1. Reversible excitation

In contrast to irreversible voltage-clamp, the induction of action potentials requires a r e v e r s i b l e, rapid perturbation of the membrane state (Fig.6,Ref.56). Electrically (47), action potentials are in general induced by a rectangular pulse of 10^{-3} sec duration in the applied voltage. This time is as fast as some of the lipid lifetimes measured by relaxation kinetics (129). Most reported ion channel open times are longer than the action potential (109; 116; 126).

Most remarkably, in contrast to ion channels, action potentials are deterministic and do not fluctuate in lifetime. Action potentials are therefore caused by deterministic forces, not by the ion channel fluctuations.

E2. Predictions

Figs. 2,3, and 7 predict that perturbation of the lipid

monolayers would result in an all-or-none response, approximately, supposed the critical range is passed. All thermodynamic lipid variables participate in the response. Reversible perturbation, called adiabatic since the entropy is constant, is propagative. The deterministic response then resembles the properties of sound.

1. The threshold of propagative all-or-none excitation is predicted by the method described in Fig.3 . The threshold units reappear: 60 mV, 1 pH unit, 1 dyne/cm for induction. Moreover, the all-or-none amplitude of the excitation, too, is of order of magnitude 60 mV, 1 local pH unit, 1 dyne/cm. Isentropic instead isothermal phase diagrams (Fig.2) are required for precise predictions.

2. The deterministic reponse of the lipid monolayers to rapid perturbation is propagated by the thermodynamic forces (Figs. 1,4). Electrical, chemical, mechanical, and thermal responses are inseparable. The absence of a pulse in temperature, in mechanical forces, or in local protonation during the electrical excitation would falsify the thermodynamic lipid mechanism of action potentials.

   A.V.Hill has reported the presence of a temperature pulse during the action potential (130;131;45;132;36). The negative temperature change during the pulse has been considered a crucial problem for the Ohmic description (49). Recently, a mechanical pulse has also been observed (133 - 136),

coupled to the electrical aspect of the excitation. All observables of the action potential are macroscopic. Clearly, neither single molecules nor purely electrical models can explain the observations any more.

The action potential does consist, as expected, of a pulse in all phospholipid surface variables investigated. Earlier, protonic control of membrane functions has already been predicted on the basis of the aqueous proton mobilities (137). Recently, propagation of protons along phospholipid surfaces has also been reported and related to semi-localised chemiosmosis (158).

Suppose then the action potential were a lipid bilayer response (55), would there be further falsifiable predictions?

Linear response theory for small lipid perturbations is not exactly applicable, since all-or-none transitions are nonlinear by necessity. Nevertheless, linearised theory has given insight into crucial properties of nonlinear physical systems, such as the behavior at threshold (70).

The linearised propagation velocity of small, reversible perturbations in any macroscopic system is known from classical hydrodynamics (138):

$$c = \sqrt{\frac{\partial \tilde{\pi}}{\partial \varrho}\Big|_S}$$

ϱ = mass surface density

The crucial prediction obtained from phase diagrams is the slowing-down of the propagation velocity in the critical regimes (Figs. 3,7), i.e., at the threshold for nonlinear all-or-none transitions. At an ideal critical point, which has not yet been identified in biological membranes, the propagation velocity is zero.

This prediction is free of adjustable parameters and independent of quantitative inaccuracies.

The observation is that subthreshold action potentials do not propagate, indeed, within experimental accuracy (139;140;141;47). Auger and Fessard have first reported the by now classical observation that the rapid propagation of action potentials, induced above a threshold of the applied voltage pulse and propagated as fast as some 10 m/sec in the squid giant axon, slows down to virtually 0 m/sec at the threshold for all-or-none responses.

4. Another prediction of the monolayer phase diagrams is the value of the propagation velocity. We find from Fig.2 isothermal, approximate values below an upper limit of ca. 20 m/sec, achieved in the more crystalline state at high surface pressures. As emphasised, these isothermal,

linearised values have to be taken with caution.

5. Nevertheless, a crucial prediction arises from the following fact: the hydrodynamic propagation velocity is much larger in three-dimensional smectics (142) than in the two-dimensional monolayer fluid. The reason for that difference is the coupled excitation of the water structure at the surface, i.e., the microscopic thickness of the layer. Three-dimensional smectic multilayers of amphiphilic molecules of some 10^2 molecular weight possess propagation velocities similar to that of sound in water: in the absence of order transitions ca. 1 km/sec. In lipid monolayers, only some cm/sec have so far been measured (82).

Saltatory propagation is therefore predicted from hydrodynamic evidence, if the two-dimensional membrane fluid is surrounded by a three-dimensional myelin sheet. The insulation by myelin from the coupled three-dimensional water structure is responsible for the increased hydrodynamic propagation velocity.

XV. Biphasic shape

The shape of the response to a reversible, rapid perturbation can also be predicted from the classical hydrodynamic equation of motion and the continuity equation. It can be obtained analytically for small perturbation. Time scale and shape of the perturbation have to be assumed in the linear case. The shape of a stationary

nonlinear pulse, in contrast, is independent from the initial perturbation (138). In this respect, a nonlinear theory for the action potential is required. For solitary solutions in physical systems (70) and their application to smectics see Ref. (143).

It is nevertheless educative to consider the linear response in a two-dimensional fluid. Local point perturbation, e.g. at a protein, is assumed. Duration and amplitude is arbitrary, but the assumed shape is monophasic and does not change sign.

The crucial question is whether the typical, biphasic shape of action potentials could arise: the depolarising pulse and the long, asymptotic hyperpolarisation of opposite, but smaller amplitude. The reconstruction of that shape required the introduction of adjustable parameters in the model of Hodgkin and Huxley (49).

Horace Lamb has actually already calculated the response to such perturbation in one-, two-, and three-dimensional fluids (138). Instead of the rectangular pulse used in most electrophysiological experiments, a Lorentz-shaped perturbation was assumed without loss of generality in the linear regime.

Fig. 8 is the result for a two-dimensional fluid, taken from Lamb's classical treatise of hydrodynamics. It shows the two-dimensional, propagating solution to that perturbation. The density S of the fluid is plotted with respect to time t at a fixed position in space. In our example, the electrode measuring the membrane excitation is placed at that position.

The linear response clearly has all characteristics of an action potential, except for the all-or-none feature. The analytical solution is somewhat involved. The physical basis of the biphasic nature of the response is simply the result of the continuity equation. No matter is added to or taken from the fluid. Any linear compression at one site of the layer must therefore be compensated by an expansion at another site. The two-dimensional integral consequently vanishes in this linear limit for any point perturbation. Any monophasic reversible point perturbation induces a biphasic response.

The density pulse per se has not yet been directly observed during action potentials. The resulting pulse in the electrical capacitance is however evident (64).

Thermodynamic principles require that the excitation is coupled. Thus, the density pulse is inseparable from a

likewise biphasic response of temperature and of the electrochemical phospholipid surface potentials. This thermodynamic principle resolves the Hill paradoxon (45):

The reversible electrical pulse implies by necessity a negative change in temperature; the temperature change is a priori reversible, apart from is dissipative components, and therefore capable not only of positive, but also of negative transients. The term negative "heat", however, is not quite adequate.

The electrical biphasic pulse is but one of many aspects of the action potential, namely the electrostatic component of the coupled electrochemical surface ion potentials, ψ:

$$\mu_i = \psi - z_i p N_i$$

$$z_i \cong 60\,mV$$

The time integral over the observed electrical potential pulse during the action potential does not vanish exactly. It has however to be taken into account that the two-dimensional spatial integral at a fixed time has to be computed and that nonlinearities may bring about aberrations in the meaning of the observed variables.

XVI. Origin of the Hodgkin-Huxley description

In view of the general acceptance of the Hodgkin-Huxley model and of the role it has played in guiding the research

on the mechanism of the action potential over several decades (22;47;124;109), the question arises which has been the experimental evidence on which this hypothesis was based. In a scientifically important review (49), A.L. Hodgkin qualifies the result of the voltage-clamp experiments actually a "disappointment", since it did not allow specific conclusions about the mechanism. The credit for the $m^3 h$ and n^4 description is given entirely to A.F.Huxley.

The origin of this descriptive model is traced back to the experiments of Cole and Curtis (64;144). Using a Wheatstone bridge, which balances the trans-membrane current by defined Ohmic resistors, electrical capacitors, and inductivities, Cole and Curtis had shown that the action potential is accompanied by a pulse in the membrane capacitance of 5% of the value at rest.

This result agrees with our expectation. Fig.2 shows that the area per phospholipid molecule changes by several per cent during all-or-none transitions in the lipid order. The lipid volume, in contrast to area, is almost constant during the polymeric transition, as can be seen from structural analysis (95). The thickness of the bilayer is thus inversely related to the area, and the capacitance changes with the square of the area.

XVII. Capacitance pulse

Today, the capacitance change of phospholipids during phase transition is established (119;145). It may be as large as

20% and allows for the smaller 5% observed even in the absence of first order phase transition. At the continuous apparent pK, e.g., capacitance changes in the order of 10% have been observed in pure lipid bilayers which show no caloric phase transition (55).

The observation of the capacitive pulse during the action potential has however been completely neglected in any of the future interpretations, because of the dramatic drop in the apparent Ohmic resistance by 4 orders of magnitude, down to the reported value (64) of 2.5 $10^4 \Omega.cm^2$.

Because of that latter observation, the action potential has almost exclusively been interpreted in terms of Ohmic resistances. The alternative approaches of Lorente de No and of Tasaki (146;27) as well as the prediction of protonic control of membrane kinetics (137) should however also be cited. Nevertheless, the one-dimensional, irreversible interpretation by trans-membrane Ohmic resistances guided the research on the mechanism of action potentials for several decades.

XVIII. Dynamic capacitive current

Let us consider the reversible membrane current more explicitly. As usual, the capacitance C is the proportionality factor relating the charge Q and the applied electrostatic voltage V .

$$Q = C V$$

Clearly, the purely capacitive current $\frac{dQ}{dt}$ consists of two parts.

$$\frac{dQ}{dt} = C \frac{dV}{dt} + V \frac{dC}{dt}$$

The first term has been considered previously. It represents the current at fixed membrane capacitance; due to a pulse in voltage, a charging current arises. We shall not discuss this term here. It is zero if the externally applied voltage is constant.

The second term has previously been neglected, an approximation which is consistent for constant capacitance. This term will be discussed for the first time (56). It describes the capacitive current at fixed voltage. This current is due to the change in capacitance and the associated "dynamic capacitive" current. This reversible, dynamic current consists of movement of charges to and from the surface, or of polarisation currents, of the dynamic dielectricum. Whatever its origin, to be derived in detail

below: at fixed voltage, the dynamic capacitance pulse is associated with a membrane current component that is proportional to voltage.

Therefore, the dynamic capacitive current is a priori indistinguishable from an Ohmic conductance.

XIX. Apparent conductivity

A Wheatstone bridge will inevitably measure the apparent conductivity $\frac{dC}{dt}$. At rest, before onset of the action potential, the capacitance is constant, ca. 10^{-6} F/cm^2. The observed apparent resistance is infinite. Therefore, it is obvious that the observed "resistance" will drop dramatically during any dynamic capacitance change. The true Ohmic resistance at rest is ca. 10^7 to 10^9 Ω cm^2. The observed capacitance pulse is 5% during 10^{-3} sec. The purely capacitive current quantitatively and by necessity appears with the following value:

$$\frac{dt}{dC} = \frac{10^{-3}\,\text{sec}}{5\% \times 10^{-6}\,\text{F/cm}^2} = 2 \times 10^4\,\Omega\text{cm}^2$$

This is within experimental accuracy exactly the value observed by Cole and Curtis. The experiments therefore demonstrate directly, that the Ohmic membrane current during the squid action potential is zero.

The Ohmic interpretation of a purely dynamic capacitive current has been a historical, crucial error in membrane theory. This error can be understood as a consequence of the traditionally one-dimensional membrane model: Dynamic capacitance has no place in one dimension.

XX. Overshoot

A dynamic capacitive current cannot be distinguished a priori from an Ohmic conductivity. A posteriori, it is distinguished by the capability of overshoot against the applied electrochemical membrane potentials. In experiments on synthetic phospholipid bilayers in the absence of chemical potential differences across the membrane, we have observed millisecond current pulses against the applied voltage (Ref.66 Fig.3). Electrode artifacts were excluded by the method of crossed electrodes. The change in sign of the current direction for crossed electrodes allows the unique assignment of the direction of the current to an asymmetry of the membrane. Clearly, pure lipid bilayers are capable of dynamic capacitive currents. The asymmetry observed predicts that the lipid bilayer is in this case not planar but curved.

Overshoot has also been observed during the squid giant axon action potential (147). Again, an Ohmic, irreversible origin of the phenomenon is excluded conclusively. It is nevertheless possible to describe the observations quantitatively by the introduction of adjustable parameters, even if such parameters are physically not meaningful.

XXI. Sodium hypothesis

At that time, the hypothesis of a sodium-specific Ohmic resistance was introduced which does conduct sodium but not potassium. There is no experiment at all which has since demonstrated the assumed a b s e n c e of potassium permeability in the presence of sodium permeability. Membrane current measurements cannot be used for that demonstration; their interpretation requires assumptions about the composition of the membrane current which were to be demonstrated. True measurements of sodium transport by isotopes (128) demonstrate, on a time scale of minutes, increase of sodium permeability during repetitive stimulation of action potentials. The absence of potassium permeability has not been observed. Moreover, these experiments do not demonstrate any ion transport during the millisecond action potential due to the slow time scale of the measurement.

The effect of tetrodotoxin, which does bind sodium and blocks or rather alters the shape of the action potential current, cannot be used either, because the sodium hypothesis had to be a s s u m e d to hold in the first place. This problem is inherent in any purely electrical measurement(47).

The more recent demonstration of voltage-induction of ion channel fluctuations in lipid bilayers in the presence of reconstituted TTX-binding proteins (109) essentially only

confirms the observation of voltage-induced ion channel fluctuations in pure lipid bilayers in the absence of proteins (Section A). It does not establish any ion channel which would not conduct potassium if it is present. The amino acid sequence of the TTX-binding "sodium channel" protein has been determined. There is however no way to tell from the sequence where the ions are moving across the membrane. Moreover, in contrast to the lipid bilayer, the proteins are only weakly fluctuating, as evident from sharp Bragg peaks, and the supposed structures appear rather static.

The idea of ion-specific membrane channels goes back to the model of Donnan (11) and was advanced by Goldman, Hodgkin and Katz (18;47). The adjustable definition of "semipermeability" of the membrane allows the description of the observed membrane potentials. Physically, however, ion-specific dissipative channel conductivities are paradox. In order to distinguish ions, entropy has to be decreased, not increased. In Section C, ion-specific transport will be derived.

III. Action potentials in absence of univalent cations

Nevertheless, the sodium hypothesis can be tested directly. Tasaki et al. have demonstrated in the squid giant axon the presence of action potentials in the absence of univalent cations (27). This falsifies the sodium hypothesis.

Hodgkin, Huxley, and Katz have introduced the method of voltage-clamp in order to prevent propagation and to

measure the membrane permeability (148). This was the goal and has been achieved. The mechanism of the action potential could not be found, because voltage-clamp is an irreversible relaxation of the membrane state, while the action potential is completely reversible. The action potential is within experimental error a purely capacitive, reversible excitation of the lipid bilayer. It propagates with the velocity of sound isentropically and therefore carries free energy along the surface.

XXIII. On ion specificity

No true measurement of exclusive movement of one cation only through ion channels has been reported except for that ion which actually represents an elementary particle: the proton.

Free energy coupling is required thermodynamically to obtain nevertheless the observed ion-specific transport. Ion-specific transport is therefore "active". It produces among other specificities the remarkable opposite sodium and potassium gradients across biological membranes including that of the squid giant axon. The specific Na,K membrane transport will be described in Section C. We shall avoid any unphysical assumption of channel specificity. To do that, we shall even assume complete non-specificity of the ion channels. Nevertheless, the active sodium/potassium transport will be predicted. The direction, magnitude, and ion-specificity will appear as the result of the normal thermodynamic response of the

phospholipid bilayer. It requires however an ion-specifically activated catalytic proton source. One such proton source is the sodium-activated sodium-potassium adenosine triphosphatase (Na-ATPase).

XIV. Unified Hydrodynamics

For the sake of a striking symmetry argument, let us forget everything said before. Let us assume that we are not aware of the capacitance pulse, the negative temperature change, and the action potentials in pure lipid bilayers in water. We only assume the validity of hydrodynamics and of the observed electromechanical coupling during action potentials.

In the last twenty years, an important development has taken place in theoretical physics. Hydrodynamics used to be limited by the presence of nonlinearities in the fundamental equations of motion, but the absence of mathematical methods for a general solution. Moreover, the description of new phenomena, such as superfluidity and liquid crystal excitations, had been rather independent from the hydrodynamics of normal fluids. Hydrodynamics appeared separated from the description of excitations in systems of other symmetries, such as crystals.

By the application of symmetry principles, Martin and Kadanoff have initiated and Martin, Parodi, and Pershan have formally completed the concept of a unified hydrodynamics for crystals, liquid crystals, superfluids,

and normal fluids (60). The beauty of unified hydrodynamics relies in the fact that, without adjusting any of the phenomenological parameters introduced, the continuous symmetries which are broken during hydrodynamic excitations allow important conclusions. These conclusions imply the allowed or forbidden coupling between the thermodynamic variables during excitation. Thus, the observations can already be analysed on the basis of symmetry principles only.

Brand and Pleiner (149;61;150) have recognised the power of the symmetry analysis for the understanding of liquid crystals and their complex phenomenology. In a series of systematic papers on nematic, cholesteric, and smectic layered liquid crystals, they made available to a great variety of systems the unified hydrodynamics and also the "macroscopic dynamics" which includes all low frequency modes. In the hydrodynamic limit, these low frequencies have in addition go to zero with long wavelength. The action potential obeys at least macroscopic dynamics, since its time scale is 10^{-3} sec and slow with respect to the microscopic time scale of 10^{-9} sec, e.g. of molecular collisions.

XXV. Smectics and membrane dimensions

In contrast to the three-dimensional smectic liquid crystals, the third dimension across a non-myelinated membrane is however m i c r o s c o p i c . This implies that no hydrodynamic variables arise with respect to the

trans-membrane direction. No physical mechanism within the trans-membrane dimension can produce the macroscopic excitation along the two-dimensional membrane surface.

In collaboration with H.Brand, I have applied these methods to the macroscopic excitations observed in the squid axon membrane. By doing so, we have unified the theory of the action potential with hydrodynamics. The physical mechanism of the action potential has been identified in microscopic detail on the basis of symmetry principles (56).

The crucial symmetry argument will be repeated here. The result will imply a microscopic interpretation not only of the action potential per se. It will describe in detail a physical mechanism for delocalised free energy coupling.

7. Electromechanical coupling

K. Iwasa and I. Tasaki (65;151;152;153;154) have observed that the squid action potential is accompanied by a rapid pulse in the mechanical force measured perpendicularly to the surface. Within experimental accuracy, the force is proportional to the derivative $\frac{d\vec{P}}{dt}$ with time t of the electrical membrane polarisation $\vec{P}$ (Fig. 9). Phenomenologically, therefore, $\frac{d\vec{P}}{dt}$ is equal to $\vec{\nabla}\vec{v}$ with a proportionality constant ς .

$\vec{\nabla}$ is the spatial gradient vector. The hydrodynamic velocity field $\vec{v}$ has to be spatially inhomogeneous in any excitation: the velocity per se is irrelevant because of the Galilei invariance. The hydrodynamic force is actually proportional to the tensor gradient $\vec{\nabla}\vec{v}$ of the velocity field. Forces appear also if the velocity only changes in a direction perpendicular to $\vec{v}$. By consequence, the proportionality factor ς is a tensor of the third rank. We do not have to know the values of these parameters for the present purpose.

XXVII. Time reversal symmetry

The phenomenological equation of the electromechanical coupling represents a physical mechanism. The equation may therefore not depend on the arbitrary choice of the direction of the spatial coordinate system. I.e., it has to be invariant under parity transformation ε_p.

Reversible physical interactions are also invariant under time reversal transformation ε_t. Irreversible interactions however result in a change of sign of the coupling tensor ς if the time axis is reversed:

$\varepsilon_t = -1$.

Under time reversal, the spatial vectors $\vec{P}$ and $\vec{\nabla}$ do not change sign per se. The time derivative $\frac{d}{dt}$ and the velocity field $\vec{v}$ however transform

like $\varepsilon_t = -1$. By consequence, the coupling

tensor is time reversal invariant: the electromechanical coupling of the action potential is r e v e r s i b l e . No irreversible mechanism can be observed during the excitation.

This is the same result which we have already obtained from the experiments of Cole and Curtis. It directly demonstrates, again, the conclusions drawn there: reversible, dynamic capacitive membrane current and isentropic, propagative mechanism of excitiation. A completely new result arises from the requirement of parity symmetry.

III. Parity symmetry

The time derivative $\frac{d}{dt}$ does not depend on the arbitrary choice of the spatial coordinate directions and transforms like $\varepsilon_p = +1$. In contrast, the vectors $\vec{p}, \vec{v}$, and also $\vec{\nabla}$ transform like $\varepsilon_p = -1$. Therefore, the system responsible for the electromechanical action potential has to possess a material property which changes sign when the space coordinates are inversed.

$$\frac{d\vec{p}}{dt} = \varsigma \vec{\nabla} \vec{v}$$

$-1 \quad (+1) \; +1 \; -1$ reversible excitation

$-1 \quad (-1) \; -1 \; -1$ macroscopic chirality

Only one physical property is known in liquid crystalline systems which obeys the parity symmetry requirement: m a c r o s c o p i c c h i r a l i t y . Only chirality changes sign under inversion of space; a left-handed helix looks right-handed in a mirror. Macroscopically ordered, chiral molecules are required as the physical basis of the electromechanical action potential. (This is also the case for rotational flows.) No individual chiral molecule can describe the macrosoopic observations.

XXIX. Identification of lipid bilayer origin

There is only one system known in biological membranes which obeys the symmetry requirement: the bimolecular lipid layer. The lipid molecules are macroscopically ordered, similar to a chiral smectic liquid crystal C* with tilt (61).

The chirality of the phospholipid molecules results from the glycerin backbone.

C-------C-------C

1 2 3

Asymmetrically, in position 3, the hydrophilic lipid phosphate head group is bound. It carries the electrical dipole moment. In position 1 and 2, the hydrophobic hydrocarbon chains are attached. The molecule is therefore chiral and not invariant under rotation. The molecular chirality may be assigned to the asymmetric carbon bonds at the 2 position.

By consequence, an ordered molecular rotation is allowed by symmetry. Most generally, it will therefore occur when the state of the lipid layers is altered, e.g. during a hydrodynamic excitation. The degree of that coupling is determined by the phenomenological components of the coupling tensors. One of these tensors is ζ . These components might be calculated from a microscopic model.

XX. The action potential

An electrical polarisation pulse is in this way associated to the mechanical density pulse during the action potential. This result generalises the thermodynamic theory of the action potential to the hydrodynamic level. The hydrodynamic theory of the action potential is also completely macroscopic. Nevertheless, and in contrast to our previous merely thermodynamic approach, it allows the following m i c r o s c o p i c interpretation:

Nerve action potentials represent a reversible hydrodynamic excitation of the macroscopic chiral phospholipid bilayer. Due to macroscopic, ordered chirality, the mechanical density pulse is breaking the continuous rotational symmetry of the chiral lipid molecules. An ordered rotation appears together with and inseparably from the density pulse. The density pulse implies the rotation of the ordered molecular electrical dipoles. It is this ordered rotation of the molecular dipoles which is observed by electrodes placed into the aqueous volumes. One of the three Cartesian components of the dipole rotation around the helical axis of the phospholipid layers is picked up by two electrodes only. If these are placed perpendicularly to the membrane plane, from the electrode view-point, the other two Cartesian components of the action potential appear as represented in the simplified design Fig. 9 .

The resulting and observed membrane current is reversible and therefore obeys the concept of local circuits (47, 27). A dissipative, open-circuit current will of course be present as in every real system. It is however irrelevant for the propagation of the excitation.

Many crucial predictions free of adjustable parameters can be derived (56). Macroscopic chirality, for instance, is directly testable. The prediction is that the electrical polarisation is geometrically coupled to a displacement along the helical axis (Fig. 9). The quantiative prediction is that the magnitude of the displacement is equal to the pitch times the fraction of a complete rotation. At least 1 degree of rotation during phospholipid phase transitions can be estimated from structural analysis (95). A pitch of 1 μm is observed in smectic liquid crystals composed of amphiphilic molecules of some 100 molecular weight. These numbers predict a displacement of magnitude 100 Å in homogeneous phase transitions, and presumably less in heterogeneous continuous transitions. The observation is ca. 10 Å displacement (65) of the axon membrane during the action potential. Most notably, the displacement is in phase with the action potential, in contrast to the mechanical force

and in agreement with macroscopic chirality.

The action potential is accompanied by a proton pulse. As we have derived above, and in detail in Ref. (56), up to 10^{11} protons can be reversibly propagated. This number arises for an axon of 1 um diameter, an all-or-none protonation, 1 msec duration, and 10 m/sec propagation velocity.

The theory of propagating excitations is of immediate relevance to the delocalisation of chemiosmosis. It results in the free energy coupling between remote locations along the membranes. The most challenging application of isentropic, delocalised membrane coupling is perhaps the function of the central nervous system. The reversibility of the action potential which we have described opens the possibility of complex but efficient free energy coupling. Receptors, action potentials, and synapses allow delocalisation, and localisation, of electrochemical, mechanoelectrical, or chemiosmotic coupling of free energy.

C. ENTROPY COUPLING

XXXI. Ion channel fluctuations and action potentials in chemiosmosis

The interplay of the thermodynamic forces (Section B) and the fluctuations (Section A) will now be considered. The forces are driving free energy along the monolayer surfaces. These surfaces may be topologically distinct, as in the impermeable bilayer, or topologically o n e , as in the fluctuating channel model I have derived. The "Möbius Band" is therefore an educative representation of transmembrane coupling (Fig. 6).

Two-dimensional fluctuations are crucial for the creation of ion pathways in the third dimension, however (Fig.4).

Previously, one-dimensional membrane theories considered the trans-membrane processes only. Very different assumptions had therefore to be made to obtain the observed chemiosmotic coupling, propagating action potentials, and ion channel fluctuations in the membrane. The receptor hypothesis excluded a priori any role of catalysis for ion channel fluctuations. Ion-specific Ohmic channels during action potentials would dissip ate but not propagate free energy. The chemiosmotic hypothesis, which we shall confirm, is distinguished from the former hypotheses by the recognition of the crucial role of the protons.

The neglect of the two-dimensional macroscopic phospholipid bilayer is common to each of these hypotheses, however. Therefore, I argued in the Introduction, the physical

mechanism for the described functions could not be found.

The consideration of the lipid bilayer responses to local catalytic activity (Fig.4) will in this section establish a thermodynamically evident mechanism for free energy coupling. This mechanism is the logical third aspect of the thermodynamic response (Fig.3): the required phospholipid perturbation.

XXXII. Compatibility with historical theories of free energy coupling

The compatibility of the phospholipid response with any one of the historically relevant theories of free energy coupling (37) demonstrates the validity of our theory:

1. The chemical intermediate hypothesis is confirmed. The previously missing energy-rich intermediate is identified. It is the (partially) protonated phospholipid molecular layer.

2. The conformational hypothesis is confirmed, too. The free energy is reversibly stored in the macroscopic order of the partially protonated phospholipid monolayers.

3. The chemiosmotic hypothesis is proven from thermodynamic principles. The localised surface electrochemical proton potential represents the most decisive intensive variable because of the proton-specificity of the phospholipid state. It is controlled by the membrane-bound "catalytic chain" (30) and justifies the "electrodic view-point" of surface coupling (40). The phospholipid-bound protons are local, but nevertheless aqueous and approximately in

equilibrium with the global proton potential in a steady state. This confirms also the experimental validity of Mitchell's chemiosmotic hypothesis (29).

III. Chemomechanical and chemiosmotic representation

Quantitatively, chemiosmotic free energy coupling to phospholipid monolayers is equal to the corresponding integral work (Fig.3)

$$\sum_i \int K_i \, dn_i$$

In the isentropic case, the efficiency of coupling is 1.

A catalytic proton source (Fig.4) performs work on the phospholipid conformation. This work reads, in the chemiosmotic representation:

$$\int \mu_H \, dN_H$$

The electrochemical proton potential μ_H is however a phospholipid variable and therefore l o c a l i s e d .

In the chemomechanical representation developed for one-dimensional polymers (34), free energy coupling reads:

$$- \int \tilde{\pi} \, dA$$

Given the surface pressure/area phase diagram in dependence on μ_H (Fig.2), the integral, reversible work performed can be calculated. We find ca. 0.1 kcal/mole protonation in most states, e.g., of the more crystalline phase (top Fig.2). This value is very small with respect to the chemical free energy of ATP catalysis, ca. 10 kcal/mole. Therefore, the phospholipid bilayers represent an inert system incapable of significant free energy coupling in these states.

Weak ion channel fluctuations and rapid propagation velocities had been obtained for such incompressible states (Sections A, B, Fig.3). We now consider the lipid response above threshold. If the critical regime is induced (cf. Section A), the fluctuations are increased in strength and the calculated free energy coupling is of magnitude

1 kcal/mole protonation

The free energy is reversibly stored in the absence of hysteresis. It can be utilised for the synthesis of an energy-rich compound. In this case, the reverse conformational transition in the phospholipids liberates the free energy.

Protonic perturbation

In the chemiosmotic representation, energy-rich protons are reversibly released from the monolayer surface. This process drives any chemical synthesis which utilises protons. One example is reversible acid-base catalysis. Another equivalent example is protonic redox coupling following the theory of Wieland (155).

In the chemomechanical representation, mechanical work is reversibly stored. An easily falsifiable prediction is that there are mechanical changes during chemiosmotic coupling.

In view of the experimental evidence on chemiosmotic coupling, protonic perturbations will only be treated in the following. Instead reviewing in detail the experimental evidence, however, my approach shall be to demonstrate

a) the compatibility of the lipid mechanism with local, semi-localised, and global theories of chemiosmotic coupling;

b) the resolution of paradoxa in previous interpretations of chemiosmosis;

c) the derivation of falsifiable experimental predictions, and the review of some experimental results obtained so far.

XXXV. Local and global coupling

The coupling mechanism is compatible with chemiosmosis as defined, on the thermodynamic level, by Mitchell. If the global aqueous volume μ is controlled, either by the electrostatic potential ψ or by the chemical proton potential $-ZpN$ at one side of the membrane. Note that only e l e c t r o c h e m i c a l potentials will be used and that the conventional attributes $\sim$ and $_H$ will be omitted for simplicity. The local surface proton potential μ_o is determined in the steady state by the aqueous equilibration between surface and bulk

$$\mu_o = \mu$$

Global chemiosmotic coupling is therefore a valid description for surface coupling in a steady state. The Pacific Ocean paradoxon (30;40), however, rules out global coupling as an efficient mechanism. Local catalytic proton production can only efficiently couple free energy if proton diffusion into the bulk volume is prevented. Otherwise, bacteria could not survive in a macroscopic aqueous volume.

We also arrive at the result that the membrane surface represents the "electrode" for the proton potential.

No free energy coupling can however arise due merely to a proton conducting system surface, or electrode, if it represented a fixed matrix. The assignment of proton conduction wires (156;157) to aqueous phospholipid surfaces

is compatible with the coupling mechanism which I am deriving. Crucially, yet, the coupling mechanism requires the conformational transition of the surface. No true phase transition is required, and the partial protonation of a heterogeneous phospholipid surface is sufficient (Fig.3).

III.I. Resolution of Williams' paradoxon

The physical problem inherent in the Williams-Mitchell controversy on local versus global coupling is the semi-conductor like proton conductivity in water and ice (137). While the coupling mechanism has to be localised, the protons are clearly hydrated and it is therefore not clear how the protons could be prevented from diffusion into the neutral, buffered bulk solution. The high proton mobility should prevent any protonic free energy coupling, in contradiction to the ubiquitous chemiosmotic coupling observed in biological membranes.

Only the lipid mechanism is capable to resolve this "proton mobility paradoxon".

Surface protons are buffered by the phospholipid lattice, not by the bulk. Bulk buffers are very weak, usually only 1 buffer site per 10^6 Å^3. Phospholipids represent the strongest buffer known in biological systems: 1 buffer site per 10^1 Å^2. This buffer is macroscopically extended along the surface of every free energy coupling membrane.

Buffering, per se, does not store free energy, however.

The proton-induced conformational change provides the storage site and, most remarkably, it also provides the s l o w t i m e s c a l e required to prevent global dissipation of protons:

$$\tilde{\tau}_{coupling} < \tilde{\tau}_{relaxation}$$

In the purely aqueous milieu, $\tilde{\tau}$ is very fast and of order 10^{-9} sec. The consequence is that protons follow immediately the phospholipid kinetics at the surface. Tsong has demonstrated phospholipid relaxation times as slow as 1 msec and 50 msec at phase transitions (129). These values are quantitatively in agreement with the "local potential" relaxations, discovered, and adequately termed, by A.V.Hill (130) using excitable biological membranes. Recently, even slower, propagating proton potentials have been reported at the surface of pure phospholipid monolayers (158).

Protons c a n n o t leave the phospholipid surface, unless the slow conformational lipid transition has occurred first. This fact provides the physical basis for the resolution of the Mitchell-Williams controversy.

XXXVII. Delocalisation

Despite the slow, dissipative movement of protons into the bulk solution, rapid, reversible propagation of protons along the phospholipid surface accompanies any adiabatic excitation. This has been demonstrated in Section B . One

example is the all-or-none action potential. The two-dimensional propagation mechanism is compatible with delocalisation of chemiosmotic coupling between remote sites at the same monolayer surface. Kell's proposal of semi-localised chemiosmosis is therefore confirmed by the lipid mechanism.

III. Redox coupling

Protonic delocalisation along the phospholipid monolayers also resolves a paradoxon mentioned by L.Ernster, related to the electronic, molecular interpretation of redox coupling at biological membranes:

How can oxidation and reduction be coupled between different molecules at remote sites, while molecular electronic states are localised within less than 50 Å ?

The Wieland theory of protonic redox coupling in aqueous hydrolysis resolves this problem, given the proton conduction at phospholipid surfaces.

$$H^+ + B^- \underset{\text{oxidation}}{\overset{\text{reduction}}{\rightleftharpoons}} A + H_2O$$

Aqueous protons produced by oxidation at one redox center, freely propagate along the two membrane dimensions, thus driving reduction at even remote sites. Reduction, like

synthesis, is actually creating forces driving proton transport and also directing transport toward that remote site.

The Wieland interpretation of trans-membrane redox coupling predicts that redox coupling has to be accompanied by proton transport. True electron transport cannot be observed, in this case. Only localised molecular electron states undergo transitions. These local transitions, observable by fluorescence spectroscopy, are coupled by protons conducted between even remote reaction centers. There is no limit in space except the membrane surface and the dissipation of a priori reversible hydrodynamic surface excitations. The stoichiometry of redox coupling, neglecting secondary phenomena, is given by the local molecular reaction and will in general be

electron transition : proton transport = 1 : 1

Experimentally, proton transport across membranes has been directly demonstrated (159) with stoichiometry approximately 1:1. Electron "transport" has not been observed directly.

The predictions of our theory are falsifiable and include: dependence of redox coupling on surface pressure; redox coupling along phospholipid monolayers; a protonic

threshold for trans-membrane redox coupling.

The Wieland-Warburg controversy of protonic versus electronic redox coupling (155;160) is almost forgotten today because of the verification by Keilin of electronic heavy metal redox centers in cytochrome (161).

XXIX. Trans-membrane coupling

So far, in this review, we have not dealt with trans-membrane coupling of free energy explicitly. We had only considered phospholipid monolayer surfaces per se. Trans-membrane coupling can only be described in this way if there exists one continuous phospholipid monolayer surface joining both sides of the bilayer membrane (Figs. 4-6).

The appearance of topological defects has been the result of Section A. The probability of these hydrophilic defects is crucially increased at the critical regime of phospholipid states.

For specific biological function, permanent uncoupling of trans-membrane gradients across the lipid bilayer channels were not useful. Therefore, biological phospholipid bilayers in the resting state should be i m p e r m e a b l e and not fluctuating, i.e., remote in the phase diagram from the critical regime. Then, the topology of the resting state is that of a smectic, liquid-crystalline and hydrophobic barrier. Residual defects are only responsible for the small resting conductivity (Section A).

The application of this result predicts a t h r e s h o l d for specific trans-membrane coupling.

XL. Threshold proton potential

Quantitatively, the threshold for trans-membrane fluxes follows from Figs. 2,3 . It is for fluid resting states of the order of magnitude 60 mV, 1 pH unit, or 1 dyne/cm as derived in Section A . Experimentally (66; 57) values between 10 mV to 400 mV had been observed. This threshold was observed for the electrochemical proton potential required for ion channel fluctuations in phospholipid bilayers. The most frequently observed threshold values were distributed around 50 to 150 mV.

More generally, I predict from Van der Waals-type diagrams a critical range of lipid states allowing for trans-membrane coupling, bound by threshold (thr) and desensitising (des) values of the forces:

$$\langle (\delta A)^2 \rangle \geqq \langle (\delta A)^2 \rangle_{thr}$$

for

$$\begin{aligned} \mu_{thr} &\leqq \mu \leqq \mu_{des} \\ \pi_{thr} &\leqq \pi \leqq \pi_{des} \\ T_{thr} &\geqq T \geqq T_{des} \end{aligned}$$

Fig. 10 presents the threshold behavior of trans-membrane fluxes expected from the phospholipid bilayer response to protonic perturbation at one side of the membrane. This prediction coincides with the reported "slips", i.e., the absence of proton flux in the presence of catalysis below a

threshold proton potential. In the biological membranes used, the threshold values ranged from ca. 60 mV to 180 mV (Refs. 162-164).

This threshold, μ_{H+}, falsifies purely molecular stoichiometries. The requirement of consideration of both μ and trans-membrane $\Delta\mu$ is obvious. The term "slip", unfortunately, invites for adjustable parameters and purely dissipative models. Such parameters are a priori only descriptive (164) and therefore not incompatible with the reversible mechanism of the critical threshold osmosis we shall derive now. Molecularly, the transport protein complex we consider will include the associated phospholipid bilayer.

The transport pathway (Fig.11) is the fluctuating, inspecific phospholipid ion channels. No trans-membrane flux has still been considered. It is now derived from the interplay between the lipid responses. The interplay of chemiosmotic monolayer coupling, of fluctuating transport pathways, and of the forces finally driving trans-membrane fluxes along the continuous phospholipid surfaces can now be appreciated, after having dealt with each individual aspect in the Sections A, B, and C above. It has been the goal of this review on free energy coupling to prove that the required interplay between phospholipid entropy, forces, and fluctuations is not just one other possible, somewhat sophisticated invention. This required interplay is the normal

thermodynamic response of the phospholipid bilayer. The only sophistication with respect to conventional thermodynamics is the two-dimensionality of the bimolecular lipid layer.

XLI. On two-dimensionality

Though, the two-dimensionality brings about completely new thermodynamic properties which cannot be conceived, except p e r d e f i n i t i o n e m , in any other thermodynamic system.

In three-dimensional systems, the thermodynamic fluctuations do not alter the nature of the system. The system is either closed, as in equilibrium thermodynamics, or it is open, as required for non-equilibrium (19). It does not matter how strong the fluctuations are.

In one-dimensional systems, as pointed out before, no macroscopic mechanism for membrane function can arise because this one dimension is microscopic.

No thermodynamic theory has so far been able to d e r i v e whether the system is open or closed. The system has to be defined first, before the thermodynamic principles can be applied. This logical requirement has by necessity lead to the introduction of adjustable definitions of the system. The meaning of thermodynamic theory is then only to demonstrate the compatibility of an adjustable definition, e.g. of semi-permeability, of a membrane. There is no physical answer to the question why the membrane is "semi-permeable."

In one-dimensional membrane theories, the possibility to derive the macroscopic membrane properties is definitely lost, since the macroscopic dimensions of the membrane along the surface are not considered.

This is the reason for the appearance of the Maxwellian paradoxon (1) and for its persistence in the discussion of the theory of free energy coupling membranes.

III. Critical osmosis

In contrast, we now d e r i v e the property of the membrane transport system as a consequence of the two-dimensional fluctuations which open and close aqueous defects above an established electrochemical proton threshold. This is the difference which will allow us to resolve the Maxwellian paradoxon and to establish the physical mechanism of trans-membrane free energy coupling.

No assumptions will be made, except for the validity of thermodynamics, of linear response theory, and of the relevance of the phospholipid monolayer phase diagrams. In particular, no assumptions of semi-permeability or ion-specificity of the trans-membrane pathway will be introduced.

Starting from complete symmetry between both membrane sides and equilibrium between surface and bulk initially (Fig.12), we demonstrate that the establishment of a transmembrane nonequilibrium (with respect to an "open" system) is the consequence of the fact that the lipid bilayer represents due to the fluctuations a "conditionally open" system. This system is then subjected to the presence of threshold activity of a local proton source (Fig.11).

Below threshold, the bilayer is impermeable and any gradient is stored in e q u i l i b r i u m of that state.

$$\mu = \mu_0 \neq \mu_0' = \mu'$$

In order to prove the establishment of the membrane gradient, however, we start from a completely symmetric state which were also in equilibrium were the system open.

$$\mu = \mu_0 = \mu_0' = \mu'$$

Note that despite of the initial symmetry the bilayer represents a closed system with respect to trans-membrane transport, due to the weak fluctuations in the resting state. With respect to macroscopic trans-membrane fluxes, it is only open if the threshold is reached. Critical osmosis in the trans-membrane dimension is the consequence.

"Critical osmosis" proceeds in four steps:

1. equilibrium

   closed

   symmetry

$$\mu = \mu_0 = \mu_0' = \mu'$$

2. local non-equilibrium

   open

   local asymmetry

$$\mu \neq \mu_{Hr} \Rightarrow \mu_0'$$

3. static head

   open

   local symmetry

$$\mu \neq \mu_{Hr} = \mu_0'$$

4. equilibrium

   closed

   global asymmetry

$$\mu = \mu_0 \neq \mu_0' = \mu'$$

1. The symmetric initial membrane state is perturbed by the local asymmetric proton source. No transport results for perturbation below threshold. The local chemomechanical excitation is propagated along the same side of the membrane or reverses the catalytic proton source.

2. Above threshold only, the closed lipid bilayer system is transformed into an open system. The ion channels, which fluctuate, provide hydrophilic surfaces across the bilayer, i.e., topological unity between both bilayer surfaces. Reversible and dissipative propagation phenomena are now directed along and across the bilayer. Trans-membrane fluxes are directed due to the catalytic asymmetry. It obeys the symmetry argument of Caplan and Kedem[39]. Following the definition by Ussing, the local, directed transport in the absence of any global trans-membrane gradient is "active". It establishes a membrane gradient globally by locally reversible or dissipative fluxes. This active gradient will however relax to zero again, supposed the bilayer membrane were open permanently.

3. No transport is driven locally anymore, once the local gradient is zero while the system is open locally. This situation establishes a static head. The local proton source cannot establish any potential larger than the one it produces locally.

4. After cessation of the activity of the enzymatic proton source below threshold, the conditionally open bilayer will again become a system that is closed. The actively transported ions cannot get back. They are trapped at the other side, and the active gradient is conserved permanently under ideal barrier conditions. This is the so-called "non-equilibrium" resting state of the membrane.

It establishes a trans-membrane gradient which is in non-equilibrium, supposed the bilayer were in the open state. Actually, it is however in the closed state and permanently stores the trans-membrane gradient in equilibrium without any further supply of free energy.

The situation is similar to a prison gate which only opens if somebody stands in front of the gate. The prison will be filled up even in the case of simple diffusion. In the case of reversibly propagating excitations, the efficiency of transport across the topological membrane channels may achieve the optimal value of one (52,53,55).

III. Steady state

The following prediction arises for the static head, i.e., the maximum membrane gradient that can be built up. If the threshold is achieved at the other, non-catalytic surface, the fluctuations render the system open permanently. No membrane gradient can be stored therefore which exceeds that threshold. By consequence, a phospholipid-dependent upper limit arises.

$$\mu' < \mu_{thr}$$

Quantitatively, the lipid limit of the membrane potential can be estimated. For initially neutral bulk pH, an effective "pK" corresponding to the threshold, and zero electrostatic potential, the static head proton potential

obeys

$$\mu' - \mu < -\mathcal{Z}(pK' - pH)$$

The electrochemical unit $\mathcal{Z}$ is ca. 60 mV at room temperature. Phospholipids of effective pK at pH 6 could only store 60 mV, while phospholipids of effective pK at pH 2 would be capable of storing 300 mV. This experimentally observed range of acid phospholipid pK values thus correctly predicts the observed regime of established trans-membrane potentials.

XLIV. Ion specificity of active transport

The ion-specificity of the established trans-membrane gradients is the result of critical osmosis in the case of ion-specifically activated local proton sources. This is to be demonstrated finally.

Although there is no evidence of absolute ion-specificity of ion channels, the observed ion-specificity of the membrane gradients of sodium and potassium is clearly established. It follows from the Second Law that ion-specific transport requires the reduction of entropy. It can only occur if work is performed. Therefore, ion-specificity of transport cannot be the consequence of ion-specific binding sites per se. These would prevent but not drive ion-specific transport. Thermodynamically, ion-specificity is therefore related to active transport, driven by local catalysis.

We start again from a completely symmetrical initial state below threshold with respect to both protons and sodium, our present example. We demonstrate in the same way as above that an active membrane gradient is established, not only with respect to protons, but also with respect to sodium if a sodium-specific proton source is present at one of the two membrane surfaces.

The mechanism of the required ion-specific activation of the catalytic proton source, e.g. Na-ATPase, is not described. It represents a black-box for our present purpose. The role of the boundary lipids for the Na-specificity of the ATPase is also not considered. The proton production in the absence of Na is assumed to be zero.

Critical osmosis now proceeds as follows:

1. equilibrium

   closed symmetry sodium potassium

$$\mu_H = \mu_{HO} = \mu'_{HO} = \mu'_H$$

$$\mu_{Na} = \mu_{NaO} = \mu'_{NaO} = \mu'_{Na}$$

$$\mu_K = \mu_{KO} = \mu'_{KO} = \mu'_K$$

2. non-equilibrium

   open asymmetry sodium no potassium

$$\mu_H \neq \mu_{H\ell_r} \Rightarrow \mu'_{HO}$$

$$\mu_{Na} \neq \mu_{Na\ell_r} \Rightarrow \mu'_{NaO}$$

3. static head

open

local symmetry

sodium

no potassium

$$\mu_H \neq \mu_{H\,glr} = \mu'_{H0} = \mu'_H$$

$$\mu_{Na} \neq \mu_{Na\,glr} = \mu'_{Na\,0} = \mu'_{Na}$$

4. equilibrium

closed

global asymmetry

sodium

potassium

$$\mu_H = \mu_{H0} \neq \mu'_{H0} = \mu'_H$$

$$\mu_{Na} = \mu_{Na\,0} \neq \mu'_{Na\,0} = \mu'_{Na}$$

$$\mu_K = \mu_{K0} \Longrightarrow \mu'_{K0} = \mu'_K$$

In the presence of only K at the catalytic site, no protons are produced. The lipid bilayer stays closed. No active potassium transport can therefore arise.

1. We start again from the completely symmetric state with respect to all variables. The membrane is closed, but would be in equlibrium even if it were open.

2. The situation is different in the presence of Na, as compared to that in the presence of K. We assumed that initially all membrane potentials were zero. Both the electrical and the chemical component of the sodium potential were thus equal on either side of the membrane.

However, sodium does activate the local proton source and in this way locally increase the electrochemical potential of both protons and sodium (Fig.12). It is crucial that the opening of the membrane is now conditioned ion-specifically.

$$\mu_{H_0} = \psi_0 - Z\rho H_0$$

$$\mu_{Na_0} = \psi_0 - Z\rho Na_0$$

The increase in the sodium potential is due to the increase in the electrostatic component of the local proton potential. This increase is the result of the positive charge of the protons. This positive charge is not neutralised at the surface despite the global electroneutrality; the proton mobility even in the structured surface water establishes the protonic control of the phospholipid state, of the chemiosmotic coupling, and also of the electrochemical sodium potential. By consequence, both protons and sodium are now driven actively across the lipid bilayer. As suggested by Fig.12,

the driving force is smaller for sodium than for protons and the potential built up should be smaller for sodium than for protons.

3. Static head is again the result of local symmetry during critical osmosis. For protons, the same value is obtained as before, since it does not matter whether the proton source was activated by a specific ion or not; it only depends on the apparent pK' of the non-enzymatic side of the bilayer membrane. The active sodium membrane potential built up is equal to the electrostatic component of the threshold electrochemical proton potential. It is smaller therefore than the static head proton gradient. Potassium has not been actively transported at all, since channel opening is sodium-specific as derived.

Quantitative predictions require to know the distribution of the electrochemical into the electrical and the chemical proton potential at the enzymatic microenvironment. Crucial predictions of critical osmosis are:

the presence of active proton transport at Na-ATPase in the same direction as Na, observed by (167);

only passive transport of potassium towards its electrochemical equilibrium potential. This is in good approximation being observed (47).

Critical osmosis is very simple from the view-point of topology. Like on a Möbius strip (Fig. 6), excitations

propagate along the surface, no matter whether this surface is actually common to both sides of the membrane. The propagated free energy cannot get back, however, once the two surfaces are topologically distinct due to insufficient fluctuations in the absence of the catalytic activity.

XLV. On Maxwell's paradoxon

From an erroneous, one-dimensional description of trans-membrane flux, critical osmosis appears as a Maxwellian Demon. Maxwell has correctly forseen the requirement for conditional opening of a gate, in order to establish a trans-membrane gradient. Such a gate would however, as he argued, violate the Second Law of thermodynamics.

This paradoxon has been repeatedly addressed, and solutions have been proposed by Szilard, Brillouin, or Feynman. In any of these solutions, the validity of thermodynamics was a s s u m e d while the one-dimensional membrane model was kept. By consequence, the Second Law was saved by assumption, not by demonstration. Only consequences of these two assumptions could be derived: the requirement of free energy supply at some stage, be it the recognition of the specific particle (165), the reopening of an irreversible door (166), or Szilards solution of an entropy increase during the process of measurement (168;63). It could not be shown that the Maxwellian paradoxon is

consistent with thermodynamics.

I conclude that the Second Law is violated because thermodynamics is applied to the wrong system. The macroscopic, thermodynamic properties of the membrane derive from the neglected macroscopic two dimensions of the surface. The only dimension previously considered is that across the membrane. It is microscopic.Because of that error only, biological membranes appear as a Maxwellian Demon. I have shown that the Maxwellian "Demon" does not violate the Second Law. It is the c o n s e q u e n c e of classical thermodynamics, applied to the two-dimensional lipid bilayer membrane.

The solution of the Maxwellian paradoxon by reversible forces in two-dimensional surfaces had already been proposed. Before the constitution of biological membranes was known, Einstein claimed that
the Second Law is consistent with experience if one considers conservative forces acting in surfaces (59).

CONCLUSION

LVI. Lipid bilayer thermodynamics

The unification of biological membrane theories is the result of the consideration of lipid bilayer thermodynamics and hydrodynamics. One example is illustrated in Figure 13, an artificial nerve axon and synapse. The normal macroscopic response of the presynaptic and postsynaptic phospholipid bilayer to the activity of catalytic proton sources is considered only. The catalysis substrates are localised in the intracellular compartment. The catalysis of acetylcholine by AChase is localised at the outer surface and zero at rest. The intracellular proton production is stationary at the Na-ATPase; it ceases however in the absence of sodium.

I have demonstrated in this review, making use of work done in collaboration with Israel Silman, Wolfgang Hanke, Ayus Corcia, and Helmut Brand, that the following responses of the lipid bilayer membrane are the consequence of first principles of thermodynamics:

Na-ATPase establishes a stationary resting state. Starting from complete symmetry, the Na-specific protonic phospholipid perturbation establishes an electrostatic membrane potential, ion-specific sodium potential, and zero trans-membrane potassium potential with the correct sign and magnitude. All-or-none electromechanical action potentials are induced by voltage, surface pressure, or local rapid proton sources and propagate along the axon in a saltatory fashion with the velocity of sound. The electrical aspect of the action potential is due to the

macroscopic chirality of the phospholipid bilayers. Synaptic transmission is like propagation reversible and can be induced electrically, if the membranes are closely attached. More generally, the electrochemical proton potential also controls synaptic transmission, as do other phospholipid variables. ACh, whether released through fluctuating ion channels or directly applied, induces the hydrodynamic excitation at the postsynaptic lipid bilayer by catalytic local perturbation. Acetylcholinesterase (AChase) perturbs the local proton potential by ca. 180 mV within 10^2 μsec (169), sufficient for propagative and deterministic postsynaptic potentials. In equilibrium after catalysis is completed, like in voltage-clamp, the electrochemical proton potential increases the reversible ion channel fluctuations and permeability of the phospholipid bilayer.

XLVII. Function of ATP and acetylcholine

ATP and ACh functions follow the same principles of entropy coupling, forces, and thermal fluctuations in the phospholipid bilayer. The lipid mechanism is compatible with local and global coupling and unifies the hypotheses of chemiosmotic coupling and receptors.

ACh-induced membrane noise and millisecond all-or-none potentials, demonstrated in the beautiful work of Katz and Miledi, are unified by the principle of thermodynamic fluctuations and forces in the lipid bilayer (115).

Action potentials are induced by electrical, mechanical, and chemical threshold perturbations of the phospholipid surface. Action potentials propagate due to the thermodynamic forces. The macroscopic chirality of the phospholipids is responsible for the rotation of the electrical phospholipid dipoles and the observed electrical polarisation pulse. The theory of the action potential is essentially a special case of the macroscopic dynamics of chiral smectic liquid crystals C* developed by Brand and Pleiner (61).

The lipid mechanism unifies the theory of the action potential with hydrodynamics on the basis of symmetry principles.

III. Control by proteins

The lipid mechanism is compatible with the previous hypotheses of protein channels or osmoenzymes, supposed the associated lipid bilayer is taken into account. Proteins are required for specific control, but the mechanism of membrane function is entirely in the bilayer.

IL. Conservative membrane function

The mechanism is thermodynamically evident and sufficient for the described functions of biological membranes. The previously unrelated membrane theories of chemiosmosis, action potentials, and ion channel opening and closing appear as the normal thermodynamic response of the hitherto neglected lipid bilayer entropy, forces, and fluctuations to local perturbation. The lipid mechanism of action potentials is reversible, capacitive, and conserves entropy. It describes the perception of electrical, chemical, and acoustical signals and the propagation of the coupled free energy to remote sites. This result immediately suggests that conservative forces may also control the function of the central nervous system.

Membranes may for good reasons be compared to Music: the instruments for performance seem difficult, but the physics is simple. Musics is in the air, like membrane excitation is in the bimolecular lipid layer.

Regine gewidmet

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Fig.1. Thermodynamic potential, force, and fluctuations of the macroscopic lipid system. The point indicates the state of the lipid bilayer after perturbation by local protein activity.

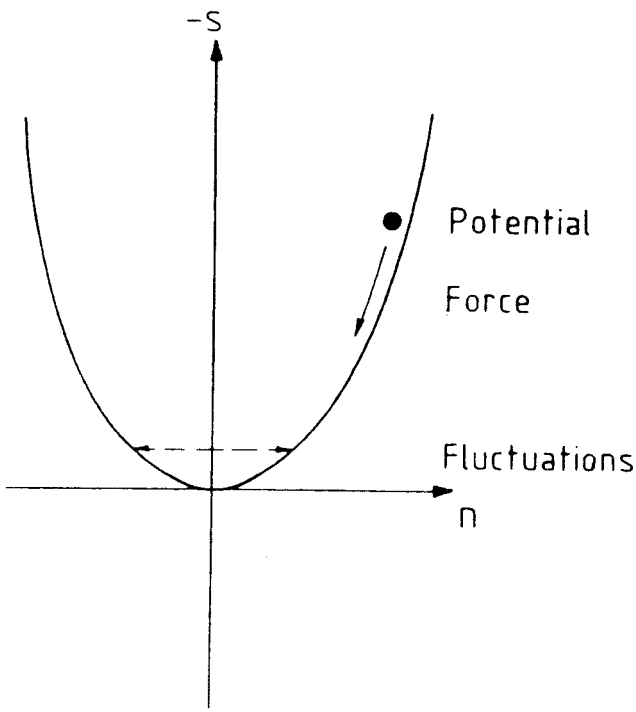

Fig.2. Phase diagram of the model lipid dimyristoyl methyl phosphatidic acid. Surface pressure is shown in dependence on area per lipid molecule, at variable global electrochemical proton potential and constant temperature 20°C. From (39).

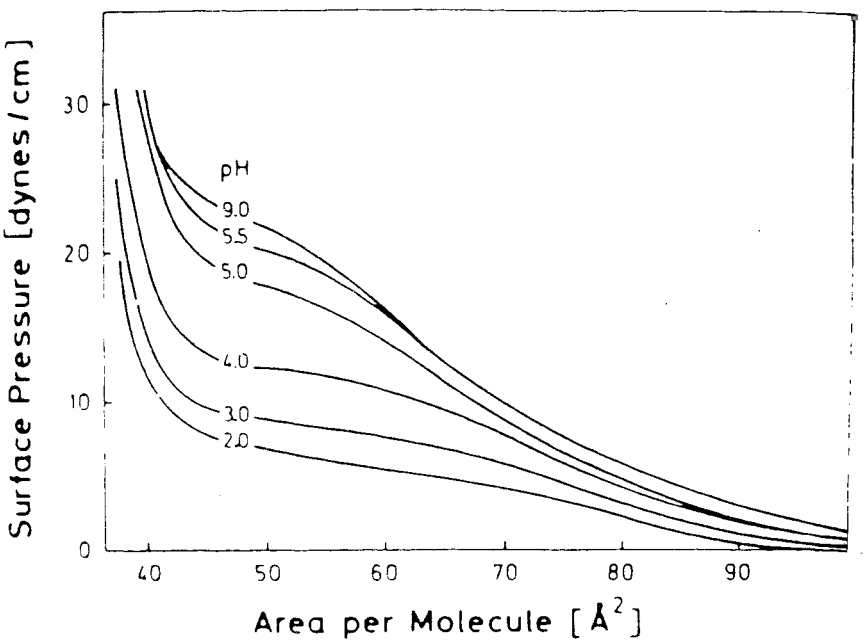

Fig.3. Quantitation of the responses to a given local perturbation: reversible work $\int K dn$ is stored in the lipid conformation; reversible excitations propagate with velocity c determined in an adiabatic phase diagram; the strength of the fluctuations is also indicated. Incompressible states show zero fluctuations and infinite propagation velocity, up to roughly 20 m/sec for Fig.2. The more susceptible states in Fig.2 are capable of storing some 1 kcal/mole protonic free energy while strong up to 10% relative area fluctuations are estimated.

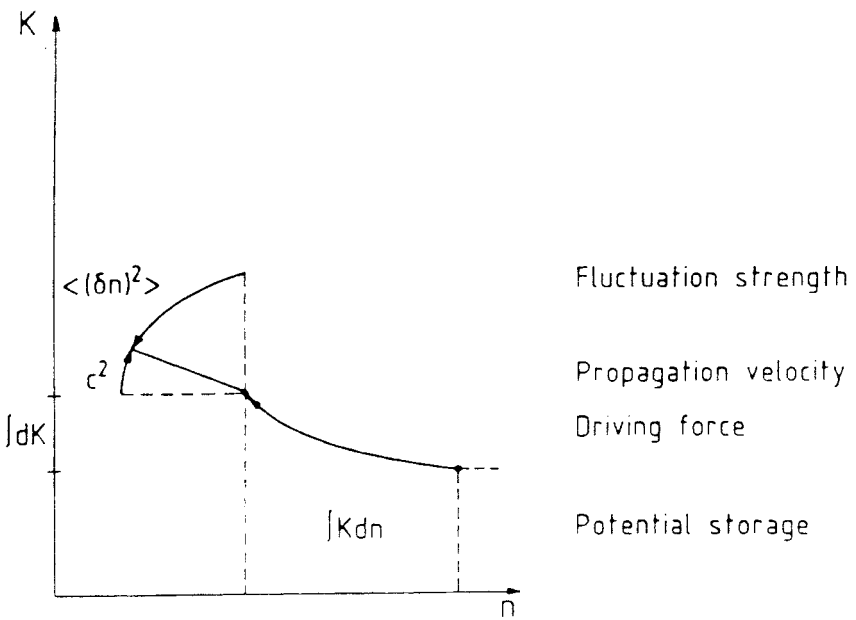

Fig.4. Phospholipid monolayer responses in a bilayer membrane after protonic perturbation by local catalysis. Free energy storage appears compatible with chemiosmosis. Forces propagate membrane excitations which delocalise the coupled free energy reversibly. The thermal fluctuations are catalytically increased and conditionally open ion channels for trans-membrane coupling.

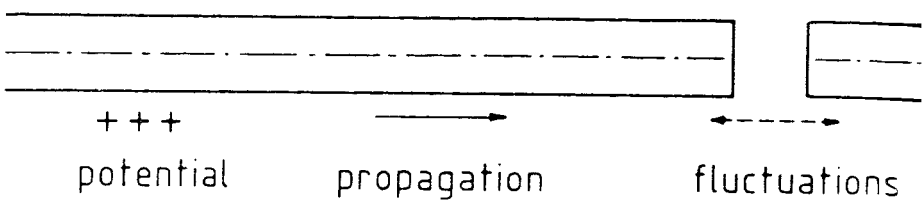

Fig.5. Electrodic view on the lipid membrane surface. Each point represents one hydrocarbon chain. Discrete defects in the hydrophobic lattice are designed. The large defects appear hydrophilic. The membrane then represents only one topological surface allowing for trans-membrane propagation of free energy.Defects are most probable in the critical regime seen in the center of Fig.2.

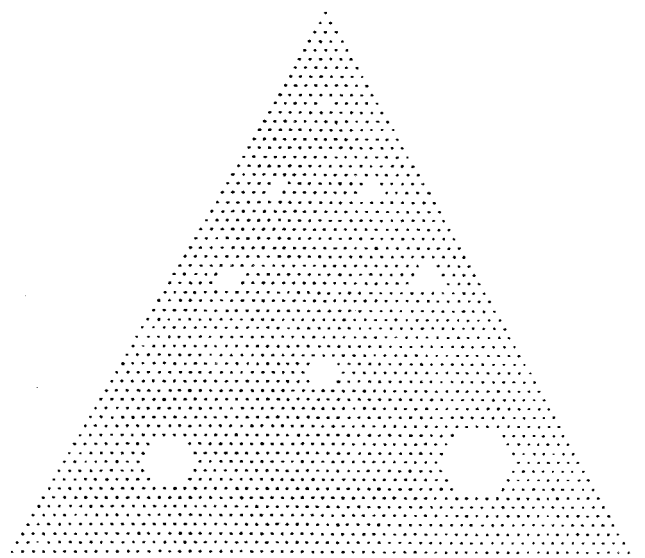

Fig.6. Möbius representation of the inner and outer membrane surface after formation of ion channel defects as in Fig.5. The thermodynamic forces drive reversible transport along and across the membrane. Remote sites of the bilayer membrane are coupled reversibly.

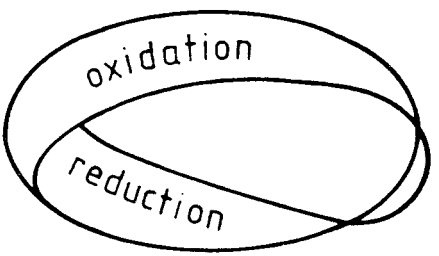

Fig.7. Subthreshold (2) and all-or-none perturbation (3), (4) of the resting state (1) ; same diagram as Fig.3.

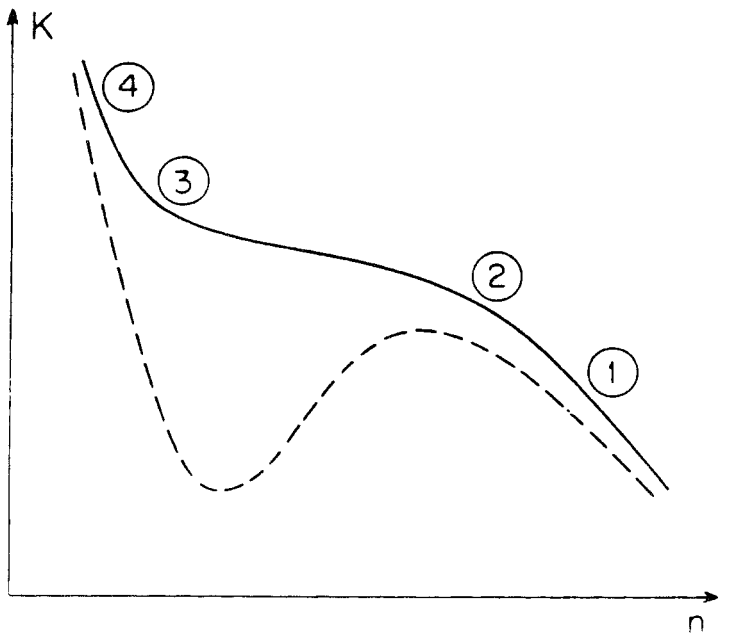

Fig.8. Linearised solution of a propagating density pulse in the two-dimensional membrane fluid after point perturbation. From (138). The biphasic response to the monophasic Lorentz perturbation is the consequence of the conservation of matter.

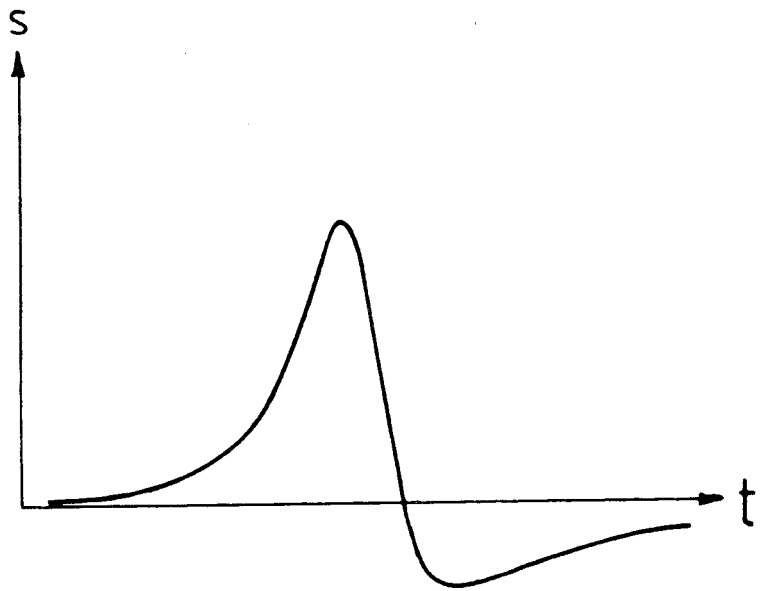

Fig.9. The rotational symmetry of the macroscopically ordered, chiral phospholipid bilayer is broken during the excitation. Macroscopic chirality explains the observed coupling between membrane displacement U and polarisation δP during the action potential of the squid giant axon. From (56;65). V indicates the velocity field of the membrane fluid.

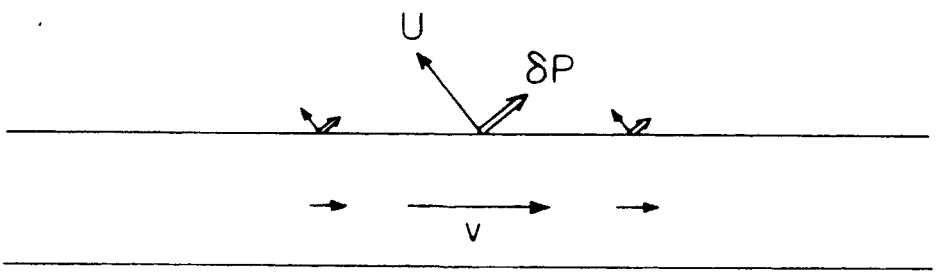

Fig.10. Threshold of the electrochemical proton potential required for trans-membrane coupling. Same as in Fig.5. The observed threshold is predicted from the protonic control of the thermal motion in the phospholipid bilayer.

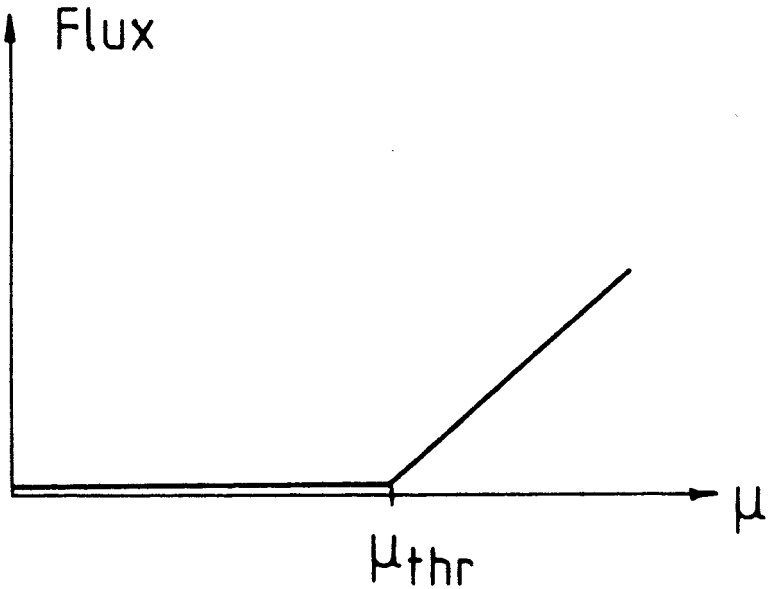

Fig.11. Critical osmosis is due to the conditionally open membrane system. The two-dimensional thermodynamic system resolves the Maxwellian paradoxon. The established membrane gradient is stored in the subcritical state of the bilayer. Rapid transmembrane coupling is reversible.

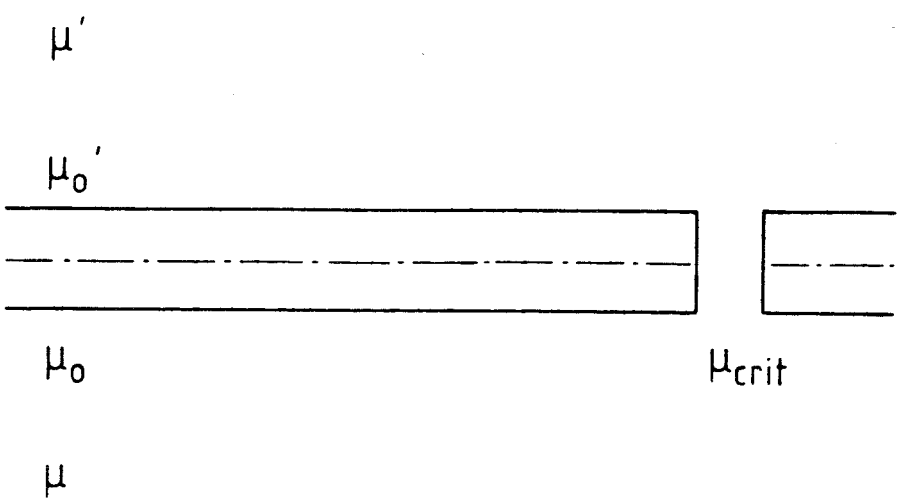

Fig.12. Ion specificity of active transport, driven by ion-specifically activated catalysis. The conditionally open membrane as in Figs. 10, 11, is now permeable to those ions only which activate the catalysis. The local proton surface potential due to the positive electrostatic component drives any activating cation toward the non-catalytic membrane side.

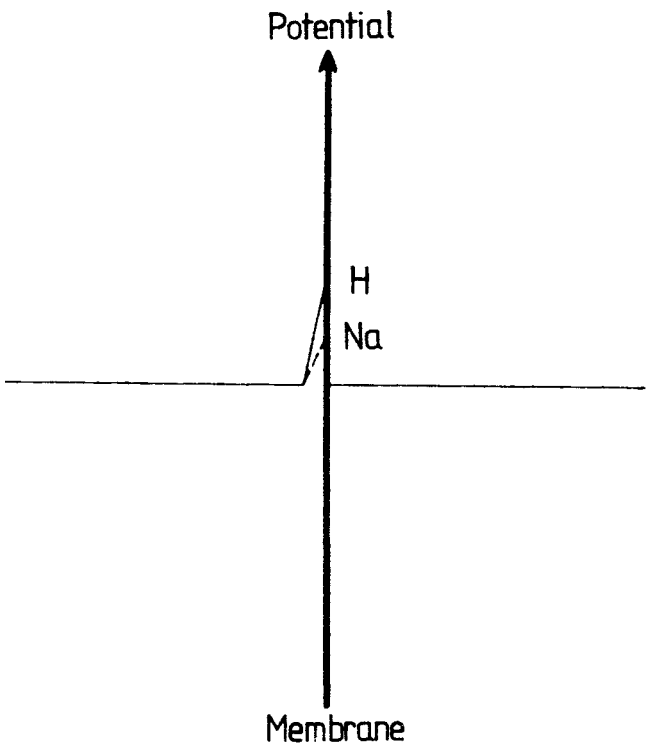

Fig.13. Artificial nerve synapse, consisting of the phospholipid bilayer and two catalytic proton sources, sodium potassium adenosintriphosphatase and acetylcholinesterase. The lipid response as in Fig. 4 establishes a negative electrostatic and electrochemical sodium potential inside, zero trans-membrane potassium potential, voltage-induced all-or-none action potentials, and acetylcholine-induced postsynaptic potentials or ion channel fluctuations. Free energy coupling, propagating potentials, and reversible channel opening are interrelated by, respectively, the entropy, forces, and fluctuations in the phospholipid bilayer. Specific protein activities control the initiation of the response. The mechanism of these membrane functions is however entirely in the lipid bilayer. The mechanism predicts the conservation of energy in the function of the biological membrane systems.

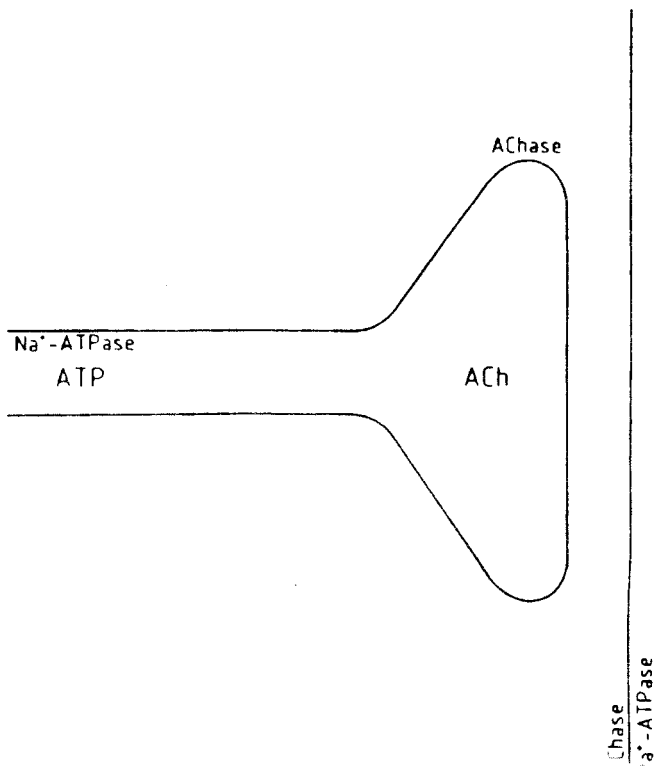

Table I.

C O M P A R I S O N OF MEMBRANE THEORIES	Lipid thermodynamics	Chemiosmotic coupling	Electrical action potentials	Molecular receptor channels
Role of lipid bilayer	+	-	-	-
proton potential	+	+	-	-
sodium channel	-	-	+	-
protein channel	-	+	+	+
Control by proteins	+	+	+	+
e.m.f.	+	+	+	+
surface pressure	+	-	-	-
Contradiction by				
threshold potential	-	+	-	-
mechanical changes	-	+	+	+
lipid ion channels	-	+	+	+
Compatibility with				
chemiosmotic coupling	+	+	-	-
action potential	+	-	+	-
ion channel fluctuations	+	-	-	+
Physical mechanism	+	-	-	-

Table II. D I C T I O N A R Y

Protein representation	Lipid representation
Chemiosmotic coupling	Proton-phospholipid coupling
Osmoenzyme	Enzyme-phospholipid complex
Lipid barrier	Subthreshold lipid state
Static head	Lipid threshold
Catalytic chain	Catalytic lipid perturbation
Translocation pathway	Lipid bilayer ion channel
Energy-rich intermediate	Energy-rich phospholipid conformation
Local coupling	Bilayer excitation
Semi-localised coupling	Propagating bilayer excitation
Global coupling	Global control of local coupling
Proton gradient	Phospholipid bilayer asymmetry
Reversible proton flux	Propagative bilayer excitation
Stoichiometric coupling	Stoichiometric catalysis
Slips	Subthreshold lipid excitation
Electron transport chain	Electronic molecular transition coupled by proton transport
Reversible chemiosmosis	Reversible phospholipid transitions
Molecular coupling	Critical osmosis
Action potential	Propagative phospholipid excitation
All-or-none excitation	All-or-none transition in lipid state
Saltatory propagation	Rapid sound velocity in smectic myelin
Electrical polarisation	Ordered rotation of molecular dipoles
Electromechanical coupling	Symmetry broken due to macroscopic chirality
Mechanical pulse	Ordered rotation and displacement along the helical axis
Negative heat	Adiabatic lipid transition
Overshoot	Adiabatic (dynamic capacitive) current
Sodium current	Sodium dependence of adiabatic current
Potassium current	Conservation of mass leading to hyperpolarisation
Capacitance pulse	Lipid bilayer density pulse
Sodium channel protein	TTX-binding protein
Voltage-induced ion channels	Lipid ion channel fluctuations controlled by voltage
Voltage-clamp	Non-propagative lipid perturbation controlling ion channel fluctuations
Receptor protein	Protein-lipid complex
Receptor conformation	Protein-induced lipid conformation
Receptor ion channel	Lipid ion channel fluctuations induced
Opening and closing channels	Reversibility of thermal fluctuations
Induction of ion channels	Increase in fluctuation strength
Desensitisation	Decrease in fluctuation strength
Point mutation of channel	Point mutation of channel-inducing polypeptide
Ion-specific channel	Ion-specific electrochemical potential
Mechanoreceptor channel	Surface-pressure induced lipid channel

SUMMARY

The role of the phospholipid bilayer membrane in free energy coupling, in summary, is to provide the physical mechanism. The thermodynamic lipid mechanism resolves paradoxes which exclude previous non-lipid interpretations.

1. Free energy supply, whether photochemical or metabolic, is the necessary cause of coupling. It is provided by specific protein catalysts. Causality excludes coupling prior to catalysis.
2. Protons play a dominant role in coupling since the phospholipid head groups are specifically susceptible to aqueous protons. The semi-conductor like proton mobility in structured surface water is responsible for the specific role of the proton potential in chemiosmotic coupling.
3. Significant free energy coupling, up to above 1 kcal/mole protons, is obtained above a proton threshold potential of order 60 mV. At this threshold potential, the surface becomes partially protonated (effective pK). Moreover, fluctuating translocation pathways in form of discrete lipid ion channels are most probable above that threshold.
4. Free energy is reversibly stored in form of reduced entropy of the protonated phospholipids. Protonation induces the transition into the energy-rich polymer conformation.
5. The free energy storing phospholipid conformation is completely reversible in the absence of hysteresis. Energy-rich protons are reversibly released from the surface when the entropy is increased reversibly, thus driving reverse catalysis.
6. The partially protonated surface is the chemical intermediate of free energy coupling. This energy-rich intermediate had not been identified previously thus leading to the chemiosmotic hypothesis.

7. Mechanical changes in membrane area and surface pressure are inseparable from the protonic aspects of chemiosmotic coupling, since all thermodynamic lipid variables are coupled in the bilayer response to catalysis at the surface.
8. Free energy coupling is localised, since the proton potential controlling chemiosmotic coupling is a phospholipid surface variable.
9. Semi-localisation, or delocalisation along the surface, of the coupled free energy is the result of the thermodynamic forces in the lipid monolayers. Action potentials, for example, propagate up to 10^{11} protons or 10^{-13} kcal protonic free energy along the membrane.
10. Global delocalisation perpendicular to the surface of the energy-rich protons is slow due to the required phospholipid conformational transition. Therefore, chemiosmosis is semi-localised. The global Mitchellian proton potential controls the surface potential. Therefore, global coupling can also be experimentally observed.
11. Chemiosmotic coupling is controlled by both μ_H and $\Delta \mu_H$. The local electrochemical proton potential μ_H controls the phospholipid conformation and ion channel fluctuations. The trans-bilayer $\Delta \mu_H$ controls the driving forces and consequent fluxes across the membrane.
12. Reversible coupling of oxidation and reduction is achieved by protons propagating along the lipid surface. Remote redox centers are coupled since no material electron transport is required.

13. Photochemical proton production at phospholipid surfaces thus couples free energy chemiosmotically. Photochemical coupling only requires the light-induced proton source and the presence of the bilayer surface and may therefore have preceeded the evolution of proteins.
14. The most rapid, diffusion-controlled proton source ubiquitous in biological membrane systems today is the protein acetylcholinesterase (AChase). Following the lipid bilayer thermodynamics described, the role of AChase is free energy-coupling proton transport by reverse chemiosmosis, the induction of propagating membrane excitation by acetylcholine, and the specific control of the opening and closing of discrete ion channels in the phospholipid bilayer.
15. ATPase couples free energy by the same mechanism as AChase.
16. Na-ATPase, which only produces protons in the presence of Na, therefore specifically drives Na together with protons across the else less specific lipid ion channels. The ion-specificity arises from the absence of channel-induction in the absence of the specific ion.
17. Ca-ATPase specifically transports Ca and protons by the same mechanism as Na-ATPase.
18. Ion-specificity of transport requires free energy and is not a property of dissipative ion channel conductivity. The threshold for lipid ion channel induction increases the ion-specificity of the catalyst.
19. Ion channels open and close due to the thermal motion in the lipid bilayer reversibly even in equilibrium. Channel induction can be specifically controlled by proteins which increase the lipid monolayer compressibilities under certain conditions.

20. Action potentials are hydrodynamic excitations of the phospholipid bilayer near all-or-none transitions. Action potentials propagate free energy with the velocity of sound. The electrical aspects of the excitation are the result of the ordered rotation of the phospholipid head group during the density pulse.
21. Hydrodynamic excitation represents the receptor mechanism for any thermodynamic phospholipid variable . Pure lipid bilayers are receptors for temperature, surface pressure, electrostatic potential, electrochemical potentials of protons, calcium, sodium and other ions. Protein activities which control the lipid surface variables are receptors for the specific agonists.
22. The reversibility of the action potential mechanism implies the fundamental reversiblity of the function of the central nervous system.
23. The mechanism of bilayer membrane function implies the establishment of asymmetry between the inner and outer compartment in the presence of bilayer asymmetry (curvature). The consideration of the macroscopic membrane dimensions in the thermodynamic theory resolves the Maxwellian paradoxon. The membrane entropy, forces, and fluctuations unify the theory of membrane function in chemiosmosis, action potentials, and ion channel formation.

On the Role of the Phospholipid Bilayer Membrane in Free Energy Coupling