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Membrane Excitability and Dissipative Instabilities

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Summary. Electrical excitation is interpreted in terms of a cooperative structural transition of membrane protomers coupled with the translocation of a permeant molecule in a non-equilibrium environment. Equations for flow of permeant and for membrane conformation are derived for the simple case of a single non-charged permeant. On the basis of a few simple physical assumptions, the theory predicts several important properties of electrically excitable membranes: the steepness of the relation between membrane conductance and potential, the presence of a negative conductance, and the occurrence of instabilities following rapid perturbations of membrane environment, giving rise to some simple cases of action potentials. Several experimental tests of the theory based, in particular, on the comparison of the conformational transition of the membrane with its changes of electrical properties are proposed. From a thermodynamic point of view, an electrically excitable membrane, in its resting state, lies beyond a dissipative instability and consequently is in a non-equilibrium state but with stable organization, a "dissipative structure" of Prigogine. Membrane excitation following a small perturbation of the environment would correspond to a jump from such an organization to another stable organization but close to thermodynamic equilibrium. It is shown how the cooperative molecular properties of the membrane are amplified by energy dissipation at the macroscopic level.

Since the beginning of the twentieth century, considerable literature has accumulated on the electrical phenomena which accompany electrical excitation in biological membranes. In particular, from the work of Cole (*see* Cole, 1955), Hodgkin and Huxley (*see* Hodgkin, 1964), Tasaki (1968) and others, a clear but largely phenomenological picture of the ionic events responsible for the electrical effects observed in the course of excitation has emerged. However, the gap between the electrophysiological observations and a physical interpretation of the molecular events which, indeed, account for these phenomena was emphasized very early (Nachmansohn, 1959). Several theories were proposed to fill this gap, but most of them were hindered by the lack of an exact understanding of the biochemical structure of excitable membranes (Nachmansohn, 1959; Changeux, Thiéry, Tung &

Kittel, 1967; Tasaki, 1969; Mullins, 1956; Hill, 1967; Adam, 1968; Gordon, 1968).

For years we have been concerned by the striking analogies which exist between membrane excitation and the control of regulatory proteins by allosteric ligands (Changeux, 1966, 1969). More precisely, *chemical* excitation was viewed as an *indirect* and thus allosteric interaction (Changeux, 1961; Monod & Jacob, 1961; Gerhart & Pardee, 1962; Monod, Changeux & Jacob, 1963; Koshland, 1963; Monod, Wyman & Changeux, 1965) between two different classes of specific membrane ligands: the chemical effector and the charged permeant (Changeux & Podleski, 1968; Changeux, 1969; Changeux & Thiéry, 1968). On the other hand, *electrical* excitation was interpreted in terms of a control of membrane permeation by the permeant ion through highly cooperative, homotropic and again *indirect* interactions (Blumenthal, Changeux & Lefever, 1970; Changeux, Blumenthal, Kasai & Podleski, 1970). In this paper we shall concern ourselves with this last aspect of membrane excitation.

A striking physical property of electrically excitable membranes is that they function in a non-equilibrium environment (Katchalsky & Spangler, 1968); in addition, their functioning *requires* this non-equilibrium situation. Indeed, electrical excitation is coupled with a passive translocation of ions through the membrane which permits a sustained propagation of the electrical signal: it constitutes an *irreversible* process which dissipates the electrochemical energy accumulated in the ionic gradient established across the membrane.

During the last few years, considerable progress has been made in the thermodynamics of open systems whose spatiotemporal organization is coupled with energy dissipation. As emphasized by Prigogine (1969), the problem of order on a macroscopic scale, in systems submitted to non-equilibrium constraints, is a problem of stability with respect to fluctuations. It was shown, for instance, that chemical systems of the type found in living organisms present a thermodynamic threshold below which processes of structuration cannot take place. The flow of energy is then insufficient to stabilize and amplify the lower entropy situations caused by fluctuations of the system around its average state. On the contrary, above this threshold point, "dissipative structures" (i.e., structures maintained by a flow of energy or matter), which are highly improbable at equilibrium, may arise and be stable.

Within the same framework of ideas, we present a point of view which incorporates both the structural and environmental aspects of membrane excitation. Our proposal is that, under physiological conditions, an electri-

cally excitable membrane in its resting state lies beyond a dissipative instability and consequently is in a non-equilibrium state but with stable organization. Membrane excitation following a small perturbation of the environment would correspond to a "jump" from such organization to another stable organization but close to thermodynamic equilibrium.

Strong emphasis is placed on a number of physical aspects of membrane structure and organization that we consider critical for the phenomenon of electrical excitation. We shall be concerned by the following, largely unexplained, properties of electrically excitable membranes (*see* Grundfest, 1966; Hodgkin, 1964).

1. They respond to brief pulses of current or rapid changes of potential.

2. The conductance or the permeability of the membrane to the selective permeant ion(s) varies with the electrical potential and this variation is extremely steep: in the squid axon "an *e*-fold increase of sodium conductance is brought about by a change of the membrane potential of the order of 5 mV" (Hodgkin & Huxley, 1952).

Voltage clamp experiments demonstrate the presence of a *negative resistance* in a given range of electrical potential. In addition, the time course of the variation of conductance under the clamp does not follow simple first-order kinetics. In their empirical fit of the squid axon data, Hodgkin and Huxley (1952) assume, to account for the change of potassium conductance, "that a path for potassium was formed when *four* charged particles had moved to a certain region of the membrane under the influence of the electrical field". If n is the probability that a single particle is in the right place, then $g_k = \bar{g}_k n^4$, where $\bar{g}_k$ is the maximum potassium conductance. ..."For the sodium channel we assumed that three simultaneous events each of probability m opened the channel to Na ...". More recent curve fittings of the same or similar data for K^+ suggest that a value of 25 is more adequate than 4 for the exponent of n (Cole & Moore, 1960). In any case, it is clear that *apparent* cooperative effects in the translocation of charged particles are essential for the generation of action potentials.

3. The response to electrical stimuli is not graded but, in general, all-or-none: there is a sharp *threshold* above which the electrical stimulus gives rise to an action potential.

Our hypotheses inspired by the data and interpretations obtained with regulatory proteins deal with two major original features of biological membranes: (1) membranes are infinite in two dimensions; and (2) the environment of a membrane *in situ* is *asymmetrical,* and electrical excitation depends on, and is controlled by, this asymmetry.

Our treatment of these hypotheses is based on the theory introduced by Hill and Kedem (1966) for transport of nonelectrolytes through a membrane lattice. It accounts for several important properties of electrically excitable membranes: (1) the steepness of the relation between membrane flux and potential; (2) the presence of a negative resistance in the current-voltage curve; and (3) the existence of instabilities following rapid perturbations of membrane environment, giving rise to some simple cases of action potentials.

Theory

1. An electrically excitable membrane is considered to be an open, isothermal lattice system placed between two baths of different electrochemical potential (environmental asymmetry; Changeux, 1969), creating a passive net flux of the permeating species, p, across the lattice.

2. The membrane lattice is made up of equivalent lipoproteic units, or protomers, specialized in the selective translocation of p. Each protomer carries at least two distinct specific sites for p, one on the inner face of the membrane, the other on its outer face. The permeant therefore both binds *and* permeates across the membrane. Transport takes place by a "jump" of p from one class of site to the other.[1]

3. At least two conformations S and R are reversibly accessible to each protomer, and both the affinity *and* the permeability of p are altered when a transition occurs from one to another conformation. As a convention, the R state will be more permeable and present a higher affinity for p than the S state.[2]

4. Cooperative interactions are established between protomers within the membrane lattice through a conformational coupling (Changeux *et al.*, 1967; Changeux, 1969). Although we favor a continuous lattice structure because it deals with a common physical property of excitable membranes,

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1 We have considered the case where the selective translocation of the permeant operates through some kind of "pore" mechanism. This particular assumption is not critical as long as the proposed mechanism deals with a selective facilitated diffusion of p. Equations have also been derived for the case of a "carrier"; the essential predictions of the theory remain valid.

2 We consider here that several conformations of the protomer preexist to the binding of p. This assumption, however, as opposed to an "induced-fit" mechanism where the conformational transition follows the binding of ligand, is not critical either; it is simply a more general formulation than the induced-fit mechanism (*see* Koshland, 1963).

there is no obligatory requirement for continuity: the protomers might be organized in dispersed clusters or patches as long as they interact in sufficiently large numbers.

5. There exists, on both sides of the membrane, a physical medium that we shall refer to as an "equilibration layer" (*see* Tasaki, 1968), in which the activity and diffusion of the ligand might be different from both that of the bulk solution and that of the membrane phase. In this layer, the concentration of ligand depends on its rate of adsorption on the membrane surface, on its rate of transport across the membrane imposed by the gradient, and on its rate of diffusion from the bulk solution.

Several of these assumptions were already formulated for chemical excitation (Changeux & Podleski, 1968). The following (mentioned above) are characteristic of electrically excitable membranes: (1) the change of affinity for the permeant in the transition of the protomer; (2) the obligatory requirement for cooperative interactions between protomers; (3) the presence of equilibration layers; and (4) the obligatory requirement for environmental asymmetry.

Equations for Flow and Membrane Conformation

We have derived equations for the case of a single permeating species, p, which, in addition, is a nonelectrolyte.

A particularly important assumption made for the derivation of these equations concerns the expression of the cooperative interactions between protomers in a lattice system. For the sake of simplicity, we have used the molecular field, or Bragg-Williams approximation (Strässler & Kittel, 1965; Hill, 1956). Following Changeux *et al.* (1967), ε being the energy required to promote one protomer from S to R when all other protomers are in state S, we express the dependence of the free energy ΔF of the transition upon the fraction $\langle r \rangle$ of protomers which are already in the R state by the simple linear relation:

$$\Delta F = \varepsilon - \eta \langle r \rangle$$

where η is a positive constant. Different treatments for cooperative interactions in a lattice system have been proposed and might alternatively be used; the general predictions of the theory will nevertheless remain valid (*see* Hill, 1956).

Fig. 1 illustrates the exchanges of matter between equilibration layers and membrane protomers according to our hypotheses.

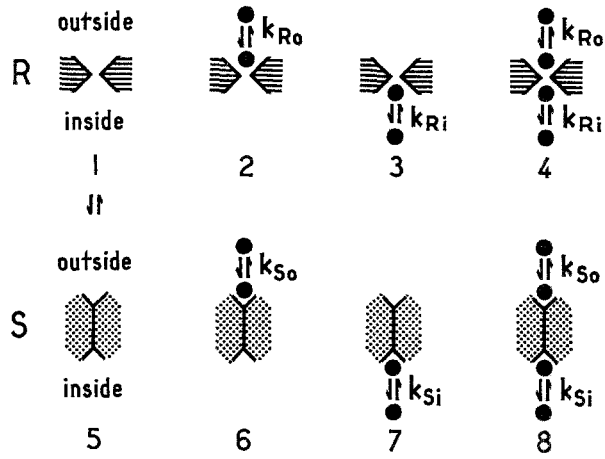

Fig. 1. The eight possible states of the membrane protomers. The transported solute is represented by a circle. In the R conformation of the protomer, the solute is both bound and transported. In the S conformation, the solute is bound but not transported. K_{Ro}, K_{Ri}, K_{So}, K_{Si} are the equilibrium constants for adsorption-desorption of the solute on the outer and inner site of protomers in the R or S state

By adsorption and desorption of p, each protomer conformation (R or S) is assumed to exist under four binding states: no binding (1 & 5), binding to the outer side (2 & 6), to the inner side (3 & 7) and to both sides (4 & 8). Translocation through the membrane occurs when a particle jumps from the outer to the inner site (steps $2 \rightarrow 3$ & $6 \rightarrow 7$). A steady state regime is established through the membrane when the translocation of p is compensated by its diffusion between the equilibration layers on both sides of the membrane and the bulk solutions where the concentration of p is kept constant. For simplicity, the kinetic equations have been written for a symmetrical protomer where the dissociation constants of p bound to its inner and outer binding sites are equal. In addition, it is assumed that p binds exclusively to the R conformation:

$$K_S/K_R = 0,$$

where K_S, and K_R are the equilibrium constants of p with S and R conformation and where K_R has the same value on both sides of the membrane. We denote the fraction of protomers in the i^{th} binding state, by,

$$x_i = \frac{N_i}{N} \tag{1}$$

where N is the total number of protomers per unit of membrane area. One has then the conservation relation:

$$x_1+x_2+x_3+x_4+x_5=1; \quad (x_6=x_7=x_8=0). \tag{2}$$

If A and B are the concentrations of ligand in the inside and outside equilibration layers, respectively, the kinetic equations now become:

$$\begin{aligned}
\frac{dx_2}{dt}&=k_aBx_1-k_dx_2-k_aAx_2+k_dx_4-k_m(x_2-x_3)\\
\frac{dx_3}{dt}&=-k_aBx_3+k_dx_4+k_aAx_1-k_dx_3+k_m(x_2-x_3)\\
\frac{dx_4}{dt}&=k_aAx_2+k_aBx_3-2k_dx_4\\
\frac{dx_5}{dt}&=k_c'x_1-k_cx_5\\
\frac{dB}{dt}&=k_p(B_o-B)+(-k_aBx_1-k_aBx_3+k_dx_2+k_dx_4)N\\
\frac{dA}{dt}&=k_p(A_i-A)+(-k_aAx_2-k_aAx_1+k_dx_3+k_dx_4)N
\end{aligned} \tag{3}$$

where A_i and B_o are the concentrations of ligand in the inner and outer bulk solution, respectively. As a convention we shall always take $B_o>A_i$. k_a and k_d are the constants of adsorption and desorption of the ligand on the protomers $\left(K_R=\frac{k_d}{k_a}\right)$, k_p the "diffusion rate constant" between the bulk solution and the equilibration layer (assuming symmetrical equilibration layers), and k_m the "diffusion rate constant" across the membrane. Making the molecular field approximation as previously mentioned, the isomerization constant of a protomer integrated in the membrane lattice is:

$$l'=\frac{k_c'}{k_c}=\exp\left[\beta(\varepsilon-\eta\langle r\rangle)\right]=lA^{\langle r\rangle} \tag{4}$$

where

$$\langle r\rangle=\frac{x_1+x_2+x_3+x_4}{x_1+x_2+x_3+x_4+x_5} \tag{5}$$

represents the fraction of protomers in the R conformation. Let us now redefine the parameters and variables in the following way:

$$\begin{aligned}
&\alpha_i=\frac{A_i}{K}; \quad \beta_o=\frac{B_o}{K}; \quad \alpha=\frac{A}{K}; \quad \beta=\frac{B}{K}; \quad \frac{k_m}{k_d}=\varepsilon; \quad k_d'=\frac{k_dN}{K}; \quad \gamma=\frac{k_pK}{k_mN};\\
&x_2+x_4=Y_o; \quad x_1+x_2=\langle r\rangle-Y_i; \quad x_3+x_4=Y_i; \quad x_1+x_3=\langle r\rangle-Y_o
\end{aligned} \tag{6}$$

and rearrange Eq. (3):

$$\begin{aligned}
\frac{dY_o}{dt} &= k_d[\beta\langle r\rangle - (\beta+1+\varepsilon)\,Y_o + \varepsilon\,Y_i] && \text{(a)}\\
\frac{dY_i}{dt} &= k_d[\alpha\langle r\rangle + \varepsilon\,Y_o - (\alpha+1+\varepsilon)\,Y_i] && \text{(b)}\\
\frac{dx_4}{dt} &= k_d[\alpha\,Y_o + \beta\,Y_i - (\alpha+\beta+2)\,x_4] && \text{(c)}\\
\frac{d\langle r\rangle}{dt} &= k_c[1-\langle r\rangle - l'(\langle r\rangle - Y_i - Y_o + x_4)] && \text{(d)}\\
\frac{d\alpha}{dt} &= k'_d[\gamma\,\varepsilon(\alpha_i-\alpha) - \alpha\langle r\rangle + (1+\alpha)\,Y_i] && \text{(e)}\\
\frac{d\beta}{dt} &= k'_d[\gamma\,\varepsilon(\beta_o-\beta) - \beta\langle r\rangle + (1+\beta)\,Y_o]. && \text{(f)}
\end{aligned} \tag{7}$$

Assuming fast equilibration of the protomers with their environment, i.e.,

$$k_d,\quad k_c \gg k'_d,$$

Eq. (7a–d) may be divided by k_d and k_c to yield a set of algebraic relations. As a result one has:

$$Y_o = \frac{[(1+\alpha)\beta + \varepsilon(\alpha+\beta)]\langle r\rangle}{(\alpha+1)(\beta+1)+\varepsilon(\alpha+\beta+2)}, \tag{8}$$

$$Y_i = \frac{[(1+\beta)\alpha + \varepsilon(\alpha+\beta)]\langle r\rangle}{(\alpha+1)(\beta+1)+\varepsilon(\alpha+\beta+2)}. \tag{9}$$

Replacing these values in Eq. (7e–f), one finds that the behavior of the membrane in response to alteration of its environment is described by a set of three equations. Two equations give the time change of the ligand concentration in the inner ($d\alpha/dt$) and outer ($d\beta/dt$) equilibration layers:

$$\frac{d\alpha}{dt} = k'_d\varepsilon\left[\gamma(\alpha_i-\alpha) - \frac{\langle r\rangle(\alpha-\beta)}{(\alpha+1)(\beta+1)+\varepsilon(\alpha+\beta+2)}\right], \tag{10}$$

$$\frac{d\beta}{dt} = k'_d\varepsilon\left[\gamma(\beta_o-\beta) - \frac{\langle r\rangle(\beta-\alpha)}{(\alpha+1)(\beta+1)+\varepsilon(\alpha+\beta+2)}\right]. \tag{11}$$

The third equation yields the fraction of membrane protomers which are in the R conformation for given values of these concentrations:

$$\langle r\rangle = \frac{1}{1+\dfrac{l A^{\langle r\rangle}\left[1+\dfrac{4\varepsilon}{(\alpha+\beta+2)}\right]}{[(\alpha+1)(\beta+1)+\varepsilon(\alpha+\beta+2)]}} \quad (\textit{equation of conformation}). \tag{12}$$

Eqs. (10)–(12) are the basic equations on which our discussion will rest.

From Eqs. (10) and (11), one deduces immediately the condition for the steady state regime $\left(\frac{d\alpha}{dt}=0,\ \frac{d\beta}{dt}=0\right)$ between the membrane and its environment:

$$J_d=J_m \quad \text{and} \quad \alpha_i+\beta_o=\alpha+\beta \tag{13}$$

with

$$J_d=\gamma(\alpha_i-\alpha)=\gamma \quad (\beta-\beta_o)$$

and

$$J_m=\frac{(\alpha-\beta)\langle r\rangle}{[(\alpha+1)(\beta+1)+\varepsilon(\alpha+\beta+2)]} \quad (\textit{equation of flow}). \tag{14}$$

Some General Predictions of the Theory

In this section we shall present some characteristic predictions of our theory for nonelectrolytes. Most of these predictions are, at least qualitatively, valid in the case of electrolytes. In a later section, these predictions will be compared to the experimental data presently available on the subject.

Variation of Flow and Membrane Conformation with Gradient

It is clear from Eqs. (12) and (14) that $\langle r\rangle$ and J_m are only functions of the concentration of p in the equilibration layers, independent of the value of the overall gradient $(\Delta\alpha)_t$ between bulk solutions. In Fig. 2a, we have plotted the flow and the membrane conformation $\langle r\rangle$ as a function of the concentration difference $\Delta\alpha=\alpha-\beta$ between the two equilibration layers.

The curves present several interesting features:

(1) Steep Variation of $\langle r\rangle$ with Gradient. Depending on the values of l and Λ, the shape of the $\langle r\rangle$ vs. $\Delta\alpha$ curve is more or less sigmoid. Below some critical value of Λ (*see* Changeux & Thiéry, 1968), an abrupt discontinuity – a phase transition – occurs. The steepness of the relation of $\langle r\rangle$ with $\Delta\alpha$ depends on the presence of cooperative interactions between protomers. When $\Lambda\to 1$, the protomers become independent and the curve of variation of $\langle r\rangle$ with $\Delta\alpha$ becomes hyperbolic. According to our hypotheses, the permeability of the membrane is directly related to the number of permeable protomers and consequently parallels the $\langle r\rangle$ function. For the general case where $\Lambda<1$, we thus predict a steep variation of permeability with gradient.

(2) Occurrence of "Negative" Permeability. For certain values of the parameters, there exists a region of the J_m vs. $\Delta\alpha$ curves where the flow of

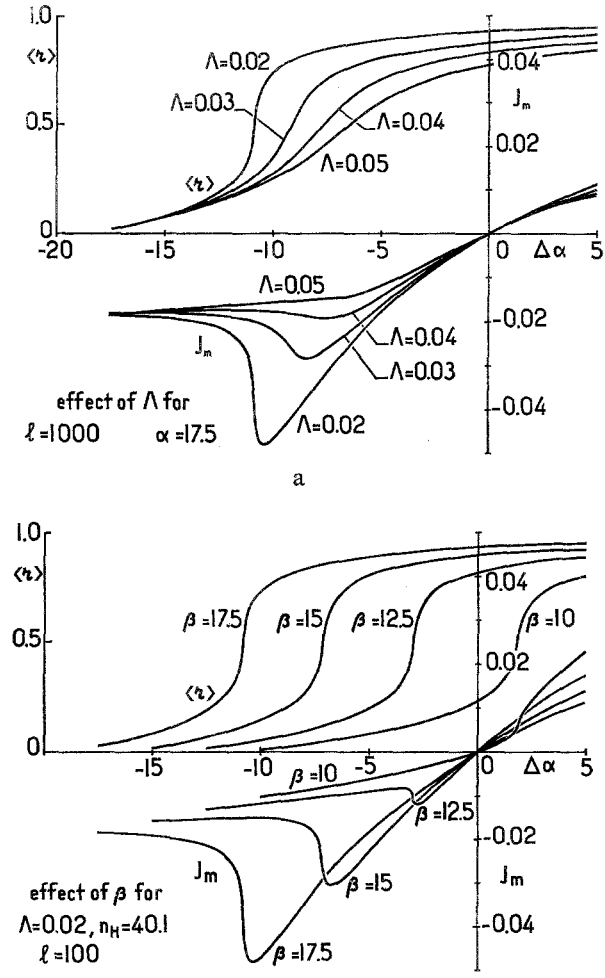

Fig. 2a and b. Variation of J_m, the flux of p across the membrane, and of $\langle r \rangle$, the function of conformation, as a function of the difference of concentration $\Delta\alpha$ between the two *equilibration layers*. The J_m vs. $\Delta\alpha$ curves are supposed to be analogous to the experimental current-voltage curves. (a) Effect of the energy of interaction between protomers (as indicated by Λ) on the shape of the $\langle r \rangle$ and J_m vs. $\Delta\alpha$ curves. The negative permeability region vanishes with decreasing cooperativity ($\Lambda \to 1$). l is defined in the text; n_H is the maximal slope of the Hill plot of the $\langle r \rangle$ function when $\Delta\alpha$ is varied. (b) Effect of the difference of concentration ($\Delta\alpha$) of p in the outer and inner equilibration layers. For each curve the concentration β in the outer equilibration layer is kept constant at the indicated value; only α is varied

permeant decreases when the gradient increases. By analogy with the "negative conductance" of the electrophysiologists, we might say here that there exists a region of "negative permeability".

The occurrence of a negative permeability region depends upon the gradient, the conformational transition of the protomers (for instance expressed by l) and the cooperativity between protomers or channels (as expressed by Λ). As shown in Fig. 3b, when the gradient vanishes, the negative permeability disappears. Presence of the negative permeability is also conditioned by the value of l. More important is the prediction that when $\Lambda \to 1$, i.e., when the protomers become independent, the negative permeability vanishes as well: a simple "rectification" of the membrane remains.

Concerning the relation between membrane cooperativity and negative permeability, two characteristic properties of our system are worth mentioning: (1) The occurrence of negative permeability does not require an abrupt "phase transition" of the $\langle r \rangle$ function. It might be associated with a graded – although steep – curve of variation of $\langle r \rangle$ with $\Delta\alpha$. From a mathematical point of view, treatment of the structural cooperativity between membrane protomers according to an oligomeric model (Monod *et al.*, 1965) might be sufficient to yield a negative permeability region.

(2) Comparison of the variation of $\langle r \rangle$ and J_m with $\Delta\alpha$ shows that the negative permeability occurs in the region where $\langle r \rangle$ varies steeply with $\Delta\alpha$.

As discussed later, this simple prediction of the theory might be tested experimentally.

Stability of Membrane Conformation Under Steady State Constraints

A characteristic and important property of the system constituted by the membrane lattice and its equilibration layers inserted between two baths of different electrochemical potential is the possibility of multi-steady state regimes for a *single value* of the overall gradient $(\Delta\alpha)_t$ established across the membrane. Indeed, Fig. 3 shows that in the conditions where the variation of $\langle r \rangle$ as a function of the gradient is very steep, or highly cooperative, there might exist three acceptable solutions of Eq. (4) which, for a given value of the constraint $(\Delta\alpha)_t = \alpha_i - \beta_o$, correspond to distinct values of ligand concentration in the two equilibration layers. Three states of regime for diffusion through the membrane are thus *a priori* accessible. To test their infinitesimal stability, we carry out a normal mode analysis. If α_s, β_s and $\langle r \rangle_s$ correspond to a set of steady state values of α, β and $\langle r \rangle$, and ω is the normal mode, the time evolution around the steady state is given by:

$$\alpha(t) = \alpha_s + (\delta\alpha)\, e^{\omega t}; \quad \beta(t) = \beta_s + (\delta\beta)\, e^{\omega t} \tag{15}$$

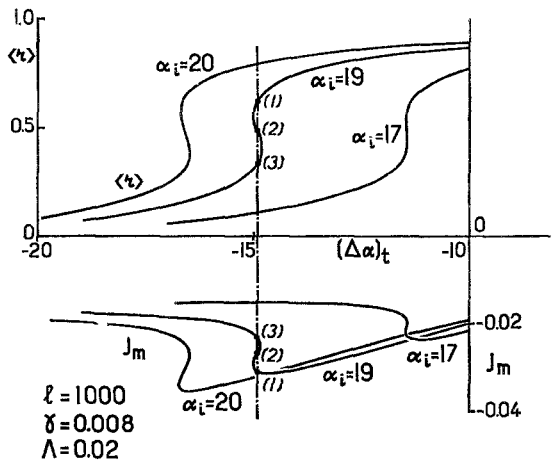

Fig. 3. Variation of J_m and $\langle r \rangle$ as a function of the overall gradient $(\Delta\alpha)_t$ between the bulk solutions. For a given value of $(\Delta\alpha)_t$, chosen in the region of cooperative change, three steady state solutions for J_m and $\langle r \rangle$ are possible. The definition of γ is given in the text

with

$$\frac{\delta\alpha}{\alpha_s} \ll 1; \quad \frac{\delta\beta}{\beta_s} \ll 1. \tag{16}$$

If all the steady states are stable, ω should always be negative. In order to see if ω might become positive and thus some of these states be unstable, we first linearize the Eqs. (10) – (12) and for simplicity consider the case $\varepsilon \ll 1$. From Eq. (12):

$$\langle r \rangle = \frac{1}{1 + l'/(1+\alpha)(1+\beta)}$$

and

$$\delta\langle r \rangle = G\left(\frac{\delta\alpha}{1+\alpha_s} + \frac{\delta\beta}{1+\beta_s}\right) \tag{17}$$

where

$$G = \frac{\langle r \rangle_s (1 - \langle r \rangle_s)}{\langle r \rangle_s (1 - \langle r \rangle_s) \ln \Lambda + 1}. \tag{18}$$

G here is always positive; only when $\ln \Lambda < -4$, are there certain values of $\langle r \rangle$ for which G becomes negative. These values correspond to an *equilibrium* phase transition of the membrane lattice, a trivial case of instability which is not dissipative, i.e., not coupled with a flow of ligand. In the following analysis, we consider the situation where $\ln \Lambda > -4$; in other words, the variation of $\langle r \rangle$ with $\Delta\alpha$ is graded. Replacing Eqs. (15) and (17)

into Eqs. (10) and (11), we get the dispersion relation for ω:

$$\left(\frac{\omega}{k'_d\varepsilon}\right)^2+\frac{\omega}{k'_d\varepsilon}\left(2\gamma+\frac{\langle r\rangle_s}{(1+\alpha_s)^2}+\frac{\langle r\rangle_s}{(1+\beta_s)^2}-\frac{G(\Delta\alpha)^2}{(1+\alpha_s)^2(1+\beta_s)^2}\right)+\left(\gamma+\frac{\langle r\rangle_s}{(1+\alpha_s)^2}+\frac{\langle r\rangle_s}{(1+\beta_s)^2}-\frac{G(\Delta\alpha)^2}{(1+\alpha_s)^2(1+\beta_s)^2}\right)=0. \tag{19}$$

The roots of this equation are:

$$\omega_1=-k'_d\varepsilon\gamma,$$

and

$$\omega_2=-k'_d\varepsilon\left(\gamma+\frac{\langle r\rangle_s}{(1+\alpha_s)^2}+\frac{\langle r\rangle_s}{(1+\beta_s)^2}-\frac{G(\Delta\alpha)^2}{(1+\alpha_s)^2(1+\beta_s)^2}\right). \tag{20}$$

As a result, if the condition:

$$\gamma+\frac{\langle r\rangle_s}{(1+\alpha_s)^2}+\frac{\langle r\rangle_s}{(1+\beta_s)^2}=\frac{G(\Delta\alpha)^2}{(1+\alpha_s)^2(1+\beta_s)^2} \tag{21}$$

is fulfilled, the system goes through a point of marginal stability. Beyond this point, ω_2 becomes positive and the steady state considered will be unstable. In order to attain this critical value, it is clear that G must be as large as possible. From Eq. (18) it is seen that $G\to 0$ both for $\langle r\rangle\to 0$ and for $\langle r\rangle\to 1$, i.e., when all the protomers are in the same conformation. On the contrary, G is maximal for $\langle r\rangle=1/2$, i.e., in the region of maximal cooperative change. Accordingly, introducing in Eq. (21) the steady state values for α and β corresponding to state (2), it is found that state (2) is unstable.

Dissipative instabilities associated with the translocation of a permeant across the membrane lattice are thus possible (prediction # 3 of Theory), even when the structural transition of the membrane is graded. Occurrence of such instabilities is conditioned by the value of the gradient and the presence of cooperative interactions between protomers. Interestingly, the presence of the instability depends also on the parameter γ which is proportional to k_p/k_m, the ratio of diffusion rate into the equilibration layer and translocation rate through the membrane. If this ratio is very high, i.e., if the diffusional barrier of the equilibration layer is negligible, Eq. (21) will never be attained. This illustrates the requirement for the equilibration layer in the presentation of the model.

In contrast, introducing in Eq. (21) the steady state values for α and β, it is found that only the two extreme steady states (1) and (3) are stable. Therefore, (2) corresponds to some kind of "threshold state". In this region

of maximum cooperativity, the concentration of ligand in the equilibration layers is predominantly controlled by the change in membrane permeability. Fluctuations around state (2) increase "consumption" of ligand by the membrane so abruptly that its "production" by diffusion from the bulk solutions can no longer compensate this effect. If we now consider states (1) and (3), small subthreshold perturbations will let the system relax to the original state, but, when state (2) is reached, the system jumps to the other stable steady state, corresponding to a different composition of the membrane in R protomers and thus to a *different permeability*. Of importance is the fact that, whatever the magnitude of the supra-threshold perturbation, the amplitude of the jump is a constant since the same steady states are always reached. The theory thus predicts the all-or-none response of the membrane to perturbations of its environment (prediction # 4 of Theory). In addition, once disturbances have been amplified locally by the instability, it is easy to see that this effect might spread over to the neighboring regions and propagate through all the membrane.

Time Course of Membrane Response to Finite Perturbations

We shall restrict our study of the time course of the membrane response to finite perturbations in the interesting domain where the values of the constants and of the overall gradient $(\Delta\alpha)_t$ permit the existence of the three stationary states. From the results discussed above, we know that two stable steady state regimes of the membrane which correspond to states of low and high permeability of the membrane are separated by a saddle point. $(\Delta\alpha)_t$ being maintained constant, a perturbation might be defined as a change of either the value of $\Delta\alpha$, or of that of $\langle r\rangle$.

From Eqs. (10)–(12) and our preceding discussion, it is clear that both quantities are closely related. Therefore one may expect that both types of perturbation lead to qualitatively identical behaviors.

Let us first analyze the time evolution of $\Delta\alpha$ in response to perturbation of the steady state value of $\langle r\rangle$.

A typical example is presented in Fig. 4 where the numerical values of the parameters selected are the following:

$$\beta_0=20;\quad \alpha_i=3.48;\quad A=0.02;\quad l=10^3;\quad \gamma=8\cdot10^{-3};\quad k'_d=10^4;\quad \varepsilon=10^{-3}. \tag{22}$$

The three steady states solutions are then:

$$\begin{aligned}
&\langle r\rangle_1=3.50 \quad \alpha_1=16.78 \quad \beta_1=6.70 \quad J_{m1}=0.0257 \quad \text{(a)}\\
&\langle r\rangle_2=4.20 \quad \alpha_2=16.46 \quad \beta_2=7.02 \quad J_{m2}=0.0283 \quad \text{(b)}\\
&\langle r\rangle_3=6.65 \quad \alpha_3=15.57 \quad \beta_3=7.91 \quad J_{m3}=0.0351. \quad \text{(c)}
\end{aligned} \tag{23}$$

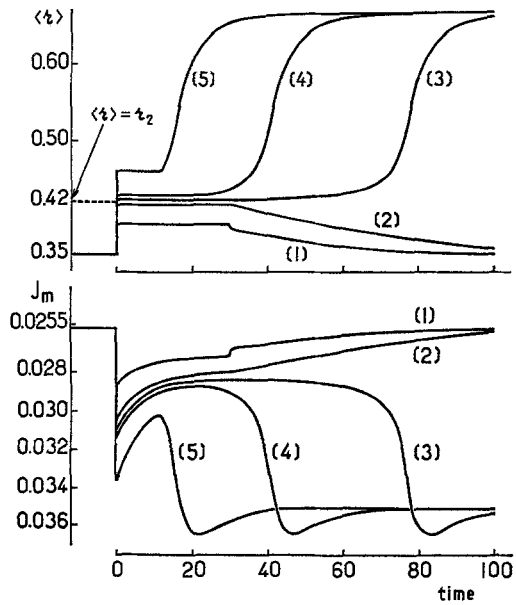

Fig. 4. Variation of $\langle r \rangle$ and J_m as a function of time after a perturbation of $\langle r \rangle$. The steady state value $r_1 = 0.35$ is altered by a rapid perturbation, finite in time and magnitude (maximum duration of 30 time units). Curves (*1*) & (*2*) correspond to subthreshold perturbations; (*3*), (*4*) and (*5*) are suprathreshold. For numerical values, *see* text

Starting with Eq. (23a) as initial conditions, we rapidly change $\langle r \rangle$ from $\langle r \rangle_1$ to $\langle r \rangle_1 + \Delta \langle r \rangle$ and maintain it at this value. Then two situations are possible (Fig. 4).

(1) *Subthreshold Perturbations.* This occurs when $\langle r \rangle_1 + \Delta \langle r \rangle < \langle r \rangle_2$ and corresponds to curves $1-2$ in Fig. 4. These curves show three distinct features: (i) the permeability increases, accompanied by a jump of the flux to a higher value; (ii) the flux decreases; the diffusion from the bulk solution no longer compensates the translocation of p through the membrane; (iii) when $\Delta \alpha$ has sufficiently diminished, a new time-dependent regime is reached. If the perturbation is then no longer maintained (a maximum duration of 30 time units has been allowed here), the system relaxes to the initial steady state Eq. (23a).

The amplitude of the maximal response of J_m is directly proportional to the amplitude of the stimulation. The response is "graded".

(2) *Supra-Threshold Perturbations.* For values $\langle r \rangle_1 + \Delta \langle r \rangle > \langle r \rangle_2$ (*see* curves $3-5$), the first stage of evolution again yields parts (a) and (b). After a given length of time, however, $\langle r \rangle$ increases rapidly, following high-order kinetics up to state (3). This fast increase of $\langle r \rangle$, owing to the

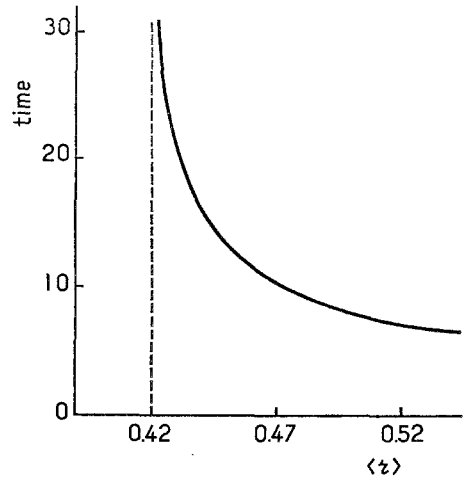

Fig. 5. Relation between the magnitude of the perturbation of $\langle r \rangle$ and the time during which it must be applied in order to observe the all-or-none response

structural cooperativity of the membrane, overwhelms the decrease of permeant gradient; as a result, the flux increases again. Under these conditions, the flux passes through a maximum, a consequence of the fact that the cooperative change in permeability occurs much faster than the change in $\Delta\alpha$ by diffusion. It is of interest that, as long as the cooperative increase of $\langle r \rangle$ has started, the system evolves spontaneously to the high permeability state. The perturbation has no longer to be maintained. As shown in Fig. 5, the time which elapses between the initiation of the perturbation and the start of the jump is directly related to the magnitude of the supra-threshold perturbation. The plot of the time elapsed before the jump as a function of the amplitude of the perturbation shows striking analogies with the strength duration curves familiar to the physiologist (Lapicque, 1909; Erlanger & Gasser, 1937).

Comparison of Theory and Experiment

The present theory is strictly valid for the case of a single nonelectrolyte species. In the majority of biological membranes, however, electrical excitation is associated with the translocation of several, often two, sometimes three, *charged* species. In spite of this difficulty, it is nevertheless instructive to compare the specific predictions of our theory with some characteristic physiological properties of biological membranes, keeping in mind that some of these properties critically depend more on a selective

mechanism of cooperative transport in a non-equilibrium environment than on the fact that the relevant permeant is charged. In subsequent papers, we will extend the theory to the electrolyte situation but with additional assumptions. In particular we will consider the influence of membrane potential on the kinetics of interaction of the ion with its membrane site, on the kinetics of the conformational transition of the protomer if one of the two states is more polarizable than the other (Hill, 1967) and on the distribution of ions in the equilibration layer on the membrane surface.

Selective Transport

An important assumption of the theory is that specific binding states of membrane protomers are involved in the process of excitation and that the selective recognition of a ligand is a process distinct from its translocation.

The distinction between outside and inside binding sites is in agreement with the experiment of Adelman and Senft (1968) and of Tasaki and his associates (Tasaki, Watanabe, Sandlin & Carnay, 1968) which demonstrate the asymmetrical behavior and thus the structural asymmetry of the squid axon membrane. The distinction between binding and permeation leads to the prediction of membrane effectors which might bind to the recognition site of the protomer without being transported and thus prevent, by *steric hindrance*, the translocation of the physiological permeant. This is probably the case for cesium ions in the giant axon of the squid: cesium ions, when added to the outside medium, block the inward current of potassium in the axon without themselves being transported (Adelman & Senft, 1968). Other ligands which cannot be considered as structurally analogous to the permeating species might also control the properties of the protomer by binding to some "*allosteric*" site distinct from the recognition site for the ion. This is possibly the case with tetrodotoxin or saxitoxin which block sodium permeability in axons (Narahashi & Moore, 1968; Hille, 1968).

Variation of Conductance with Potential

Although our equations have been derived for a non-charged permeant, it is nevertheless of interest to investigate what are the characteristic predictions of the theory for the relation of flux (J_m) with the chemical gradient ($\Delta\alpha$), assuming for the time being that such a relation parallels the relationships between electrical current and membrane potential in the electrolyte case.

As mentioned above, the curves given by the theory show a striking similarity of shape with the current-voltage curves measured with a variety

of biological preparations (Grundfest, 1966). We have mentioned that, according to our theory, the negative permeability region disappears when the electrochemical gradient across the membrane is abolished. In fact, when a squid giant axon is internally perfused with a medium having the same ionic composition as its external environment, the negative conductance region of the current-voltage relationships is no longer present (Baker, Hodgkin & Shaw, 1961).

Our theory is also in agreement with the observations of Dodge and Frankenhaeuser (1959) on the "node of Ranvier" that the variation of external concentrations of sodium promotes a change in the current-voltage curve without changing the shape of the curve of variation of sodium permeability with potential. Their experimental results can be fitted remarkably well by theoretical curves similar to those shown in Fig. 2, assuming the sodium permeability to be proportional to our $\langle r \rangle$ function.

Instabilities

The theory predicts that, for given values of the constants and of the constraint, perturbation of the membrane environment or of membrane structure might give rise to an all-or-none response. Excitation is viewed as a "jump" between two stationary states of the membrane with different permeabilities for the same permeating species. Such a property is highly reminiscent of some simple cases of membrane response to a small variation of electrical current or potential: for example, the abrupt rectangular depolarization observed with a variety of biological membranes under special conditions of environment (*see* Tasaki, 1968, p. 105; Grundfest, 1966) or the resistive action potentials of some artificial membranes (Mueller & Rudin, 1968).

Interestingly the kinetics predicted by the theory (Fig. 4) are strikingly similar to those obtained with various membranes after a brief pulse of current (or potential).

Our theory can easily be extended to fit the more complicated but classic case of a transient action potential and, more particularly, to explain the spontaneous reestablishement of the resting potential after the initial depolarization. At least *two different permeating* species will then be required.

From a structural point of view, it is of interest that the presence of equilibration (or "unstirred") layers have already been postulated for years in the case of the axonal membrane (*see* Tasaki, 1968, p. 46). Fixed charges might play an important role in controlling the diffusion of electrolytes in the biological case (Mauro, 1962).

Cooperative Structural Transition

An important aspect of our theory is the assignment of the changes in the electrical parameters of excitable membranes to a characteristic modification of membrane structure. These structural changes which have not yet been identified unambiguously might involve both the protein and/or the lipidic moiety of the protomers, their *motion* as well as their *configuration*. However, the experimental data obtained by various workers (Cohen, Keynes & Hille, 1968; Tasaki *et al.*, 1968; Tasaki, Carnay, Sandlin & Watanabe, 1969; Carnay & Barry, 1969) on optical changes accompanying the propagation of action potentials suggest that such an identification should be possible in the near future. Particular emphasis is given in our approach to the distinction between the flux equations and the conformation equations. Experimentally these two functions should be distinct; moreover, as illustrated in Fig. 2, the theory predicts that the function of conformation should vary steeply with the gradient in the conditions where the negative permeability or conductance is observed. Such an experimental test of the theory would be to some extent analogous to the comparison between state and binding functions suggested by one of us (Changeux & Rubin, 1968) as a clue for the characterization of allosteric transitions in regulatory enzymes.

In order to explain membrane cooperative effects, we have proposed a structural, or conformational, coupling between protomers or channel-carrying elements. First of all, the precise distribution of the protomers within the membrane is not critical as long as the protomers interact. Although we favor a lattice model because it deals with an original structural property of biological membranes, as mentioned above, alternative mathematical treatment of the cooperative interactions between neighboring protomers, even an oligomeric model, might give similar results. We should mention, in relation to this point, that repeating units organized in a more or less regular lattice structure have been well identified in several axonal and synaptic membranes. In particular, such a structure is present in membrane fragments which originate from the innervated excitable face of the electroplax but is absent in fragments which originate from the non-innervated inexcitable one (Benedetti & Changeux, *unpublished observations*).

A serious alternative to our structural hypothesis is that the apparent cooperativity arises solely from a long-range, indirect *electrical* coupling. The effects would be "collective" rather than cooperative. A critical prediction of such an electrical coupling is that the cooperativity would be strictly determined by the absolute value of the potential and would be

less dependent upon the nature of the ion transported. In fact, this does not seem to be the case with biological membranes (Hodgkin, 1964). A more direct proof for structural cooperativity would be to show that cooperative effects are still present at *constant electrical potential* when only the ionic (chemical) gradient is changed. This seems to be the case for potassium ions with the electroplax (*unpublished experiments*). Moreover, the demonstration of a structural coupling might come from the performance of a *desensitization* experiment, analogous to the desensitization of regulatory enzymes (Changeux, 1961; Gerhart & Pardee, 1962): adequate chemical treatments of the membrane might selectively alter the nearest-neighbor interactions between ionophores. Correlatively, the observed cooperative effects would be impaired and the all-or-none response might become graded.

In any case, accounting for the very high value of the electrical fields established across the membrane, the occurrence of some long-range electrical effects, superimposed to the structural effects, might have to be seriously considered.

Link Between Membrane Excitability and "Dissipative Structure"

In this section, we would like to make more explicit, from the thermodynamic point of view, the relation between the properties of a membrane under equilibrium and non-equilibrium conditions. Therefore it might be worthwhile to make a brief comparison with other examples of unstable systems in biology. Indeed, it has been reported (Prigogine, Lefever, Goldbeter & Herschkowitz-Kaufmann, 1969) that some enzymatic systems seem to function beyond a dissipative transition under physiological conditions. More precisely, instabilities are present beyond which part of the energy dissipated by the chemical processes can be transformed into internal order, in time, in space, and from the functional point of view. These phenomena illustrate how the *microscopic* properties of enzymes, (e.g., activation, inhibition, cross feedback or autocatalysis, etc.) can be magnified by the flow of energy into order at a *macroscopic* level. The concept of "dissipative structures" was therefore introduced by Prigogine (1969) to describe this type of structuration processes which, at least to some extent, might be considered as the "building steps" of biological order.

In the same way, the results reported here indicate how the cooperative *molecular* properties of membrane units can be *amplified by energy dissipation* and account for the phenomenon of membrane excitability. If we go back to the behavior of the system under equilibrium conditions, Eq. (12) which gives the conformational state of the membrane reduces immediately

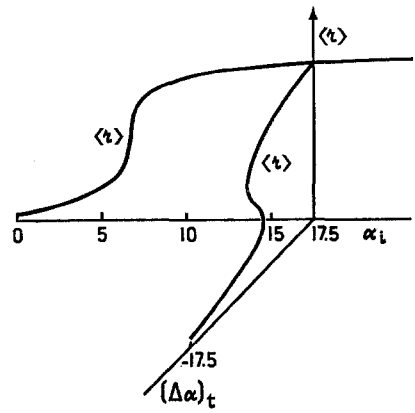

Fig. 6. In the plane corresponding to thermodynamic equilibrium (i.e., for $\beta_0=\alpha_i$), we have plotted $\langle r\rangle$ as a function of α_i, the normalized concentration of p in the inner bulk solution. Only one value of $\langle r\rangle$ is then accessible to the membrane for a single value of α_i. In a plane perpendicular to the plane of the figure, we have plotted $\langle r\rangle$ as a function of the overall gradient $(\Delta\alpha)_t$ for a given value of α_i. The figure shows how the behavior of the system is modified when the membrane is brought into environmental asymmetry

to the one derived by Changeux *et al.* (1967):

$$\langle r\rangle = \frac{1}{1+\dfrac{l\Lambda^{\langle r\rangle}}{(1+\alpha)^2}}$$

with $\alpha=\beta=\beta_o=\alpha_i$.

One sees immediately then, for example, within the framework of numerical values considered here for Λ ($\Lambda<\Lambda_c$), that at equilibrium no multiple states for $\langle r\rangle$ are possible for a single value of α, and consequently there is no threshold state or instability. As shown in Fig. 6, a multivalued $\langle r\rangle$ function appears here as the result of the "functioning" of the system inserted in a concentration gradient. The system possesses the freedom to leave the equilibrium plane where $\beta_o=\alpha_i$ and to find new working conditions more adapted to its environment. As a result we see that when the deviations of the overall gradient from equilibrium are small, the conformational curve $\langle r\rangle$ corresponds to an extrapolation of the equilibrium situation. One may therefore say that the membrane state lies then on the "thermodynamic branch".[3] On the contrary for bigger values of the gradient, the

3 This is in agreement with the well-known experimental fact that, at the peak of the spike, the electrochemical potential difference for sodium ions across the membrane is not far from zero.

membrane, in our model, exhibits a low conductance and is in its resting state. The membrane organization or conformation would then lie on the other branch which corresponds to a "dissipative structure". This again exemplifies the fact that the constraints imposed by the outside world on biological systems bring them to a state where their properties can no longer be understood solely on the basis of an extrapolation of the equilibrium situation (for more detail, *see* Prigogine & Nicolis, 1967; Lefever, Nicolis & Prigogine, 1967; Prigogine & Lefever, 1968; Lefever, 1968). Here we see that the resting state of the membrane corresponds to a molecular organization which is separated from its excited state, obtained by gradual deviation from equilibrium, by a discontinuity; a jump across this discontinuity following small perturbations constitutes the membrane excitation process. Since such a process is based on the coupling of a cooperative structural transition of the membrane with a downhill translocation of ions, we might qualify membrane excitation as an "assisted phase transition".

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