

# Lawrence Berkeley National Laboratory

## Recent Work

### Title

APPLICATION OF ELECTROCHEMICAL KINETICS TO PHOTOSYNTHESIS AND OXIDATIVE PHOSPHORYLATION: THE REDOX ELEMENT HYPOTHESIS AND THE PRINCIPLE OF PARAMETRIC ENERGY COUPLING

### Permalink

<https://escholarship.org/uc/item/0xz8f87w>

### Author

Tributsch, Helmut.

### Publication Date

1971-05-01

To be published in Journal  
of Bioenergetics

RECEIVED  
LAWRENCE  
RADIATION LABORATORY

UCRL-20832  
Preprint e2

DOCUMENTS SECTION

APPLICATION OF ELECTROCHEMICAL KINETICS  
TO PHOTOSYNTHESIS AND OXIDATIVE PHOSPHORYLATION:  
THE REDOX ELEMENT HYPOTHESIS AND THE PRINCIPLE  
OF PARAMETRIC ENERGY COUPLING

Helmut Tributsch

May 1971

AEC Contract No. W-7405-eng-48

**TWO-WEEK LOAN COPY**

*This is a Library Circulating Copy  
which may be borrowed for two weeks.  
For a personal retention copy, call  
Tech. Info. Division, Ext. 5545*

48  
LAWRENCE RADIATION LABORATORY  
UNIVERSITY of CALIFORNIA BERKELEY

UCRL-20832

e2

## **DISCLAIMER**

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

APPLICATION OF ELECTROCHEMICAL KINETICS TO PHOTOSYNTHESIS AND  
OXIDATIVE PHOSPHORYLATION: THE REDOX ELEMENT HYPOTHESIS  
AND THE PRINCIPLE OF PARAMETRIC ENERGY COUPLING

Helmut Tributsch

Laboratory of Chemical Biodynamics  
Lawrence Radiation Laboratory  
University of California  
Berkeley, California

Running Title:

REDOX ELEMENT HYPOTHESIS AND PARAMETRIC COUPLING

Abstract

It is suggested that the transfer of electrons within the biological electron transfer chain is subject to the laws of electrochemical kinetics, when membrane-bound electron carriers are involved. Consequently, small tightly bound molecular complexes of two or more electron transfer proteins of different redox potential within an energy transducing membrane, which accept electrons from a donor at one membrane surface and donate it to an acceptor at the other, may be regarded as real and functioning molecular redox elements, which convert the free energy of electrons into electrochemical energy. Especially, the transfer of an electron from excited chlorophyll to an electron acceptor can be looked upon as an electrochemical oxidation of excited chlorophyll at such a complex. In this reaction the electron acceptor complex behaves like a polarized electrode, in which the electrochemical potential gradient is provided by a gradient of redox potential of its constituents.

Calculations and qualitative considerations show that this concept leads to a consistent understanding of both primary and secondary reactions in photosynthesis (electron capture, delayed light emission, ion transfer, energy conversion) and can also be applied to oxidative phosphorylation. Within the proposed concept, ion transfer and the development of ion gradients have to be considered as results of electrochemical activity--not as intermediates for energy conversion. For energetic reasons, a non steady state, periodic energy coupling mechanism is postulated which functions by periodic changes of the

capacity of the (electrochemically) charged energy transducing membrane, during which capacitive surplus energy is released as chemical energy. Energy transducing membranes may thus be considered as electrochemical parametric energy transformers. This concept explains active periodic conformation changes and mechanochemical processes of energy transducing membranes as energetically essential events, which trigger energy conversion according to the principle of variable parameter energy transformers.

The electrochemical approach presented here has been suggested and is supported by the observation, that with respect to electron capture and conversion of excitation energy into electrochemical energy, the behaviour of excited chlorophyll at suitable solid state (semiconductor) electrodes is very similar to that of chlorophyll in photosynthetic reaction centers.

### Introduction

The present study started with an investigation of the mechanism in photosynthesis by which electrons from excited chlorophyll molecules are effectively captured. This process is especially remarkable in that (a) electron transfer occurs from the shortlived excited singlet state of chlorophyll, (b) it occurs with high quantum efficiency, and (c) that reverse reactions of the reduced acceptor with the oxidized chlorophyll molecule are effectively suppressed. In these respects chlorophyll sensitized reactions in photosynthetic reaction centers are clearly different

from chlorophyll reactions in homogeneous solution: It is true that excited chlorophyll a in a homogeneous solution can reduce suitable compounds in an electron transfer reaction. This has been shown, for example, to occur with chlorophyll a and quinone in isobutanol.<sup>1</sup> However, the longlived chlorophyll triplet state is involved in this electron transfer reaction, and the resulting reduced intermediate (semiquinone) is reoxidized by chlorophyll<sup>+</sup> in a diffusion controlled reverse reaction.

In an attempt to explain the unusual features of the primary electron capture process in photosynthesis, the hypothesis was made that the electron transfer reaction of excited chlorophyll in photosynthesis may be an electrochemical oxidation of excited chlorophyll at an acceptor which acts like an anodically polarized electrode in that it captures the electron in a gradient of electrochemical potential.

Experiments designed to test this hypothesis were successful in that they showed that excited chlorophyll molecules can be oxidized in electrochemical reactions when solid state systems with suitable energy band structure are chosen as electrodes.<sup>2</sup> In our experiments, single crystals of semiconductors (e.g., ZnO) with a large energy gap were used which provided a conduction band for electron capture, which coincided energetically with the excitation level of chlorophyll. Excited chlorophyll was found to exchange electrons with the conduction band of the semiconductor. When the semiconductor was used as an electrode in an electrochemical cell and a small positive voltage (+0.5 Volt) was applied, these electrons could be conducted away from oxidized chlorophyll and a reverse reaction of the electron prevented.

Figure 1 shows, as an example, a spectrum of the electron current, which is injected by excited bacteriochlorophyll into a single crystal semiconductor electrode. In Figure 2 the dependency of the photocurrent on the electrode potential is depicted.

Interestingly, there is still a positive photocurrent, when the external potential is disconnected. In this case there is still a small electrochemical potential gradient in the electrode surface, which produces a directed electron current. Under these conditions, photochemical energy of chlorophyll redox reactions is converted into electrochemical energy.

The quantum efficiency of electron capture from chlorophyll a was estimated to reach 2.5% under favorable conditions. In the presence of suitable reducing agents the quantum efficiency exceeded 10%.

These electrochemical investigations have shown in particular that photochemical redox reactions can be controlled with the help of electrodes. Electrons can effectively be captured from the excitation level of chlorophyll, and unidirectional electron flow can be produced when an electrochemical potential gradient within a solid state acceptor system is provided to transfer the accepted electrons away from oxidized chlorophyll. This has to occur in a time which is comparable with the lifetime of the excited molecule. The relaxation time for an electron in a semiconductor electron acceptor is sufficiently short to meet this condition.

#### Are Electron Transfer Reactions in Biological Electron Transfer Chains Subject to the Laws of Electrochemistry?

Electrochemical reactions of excited chlorophyll molecules were found to be very similar to reactions of chlorophyll in photosynthetic



reaction centers. Excited chlorophyll molecules at a positively polarized semiconductor electrode stimulate a unidirectional electron flow with high efficiency, act as photon-powered pumps for the transfer of electrons from a donor to the acceptor electrode, i.e. they are able to convert excitation energy into electrochemical energy.

These results prompted the suggestion that chlorophyll reactions in photosynthesis may be considered and described as electrochemical reactions.

A closer look at the energy schemes of photosynthesis (Fig. 3) confirms this assumption: Most of the electron transfer molecules within the electron transfer chain are membrane-bound, and appear to exist in a well defined arrangement. Electron transfer reactions, however, in which membrane-bound electron transfer molecules are involved, will in general be accompanied by the appearance of electric potentials, for it cannot be expected that statistical charge compensation within the membrane will be sufficiently fast. Electric potentials, of course, contribute to the free energy of electrons,

$$dG = (\mu_{ch} + F\psi)dn \quad (1)$$

( $\mu_{ch}$  = chemical potential,  $F$  = Faraday constant,  $\psi$  = electric potential,  $dn$  = number of moles) and thus influence the rate (and equilibrium constants) of electron transfer reactions:

$$k \sim \exp(-\Delta G/kT)$$

Consequently, it is necessary to apply electrochemical kinetics to describe electron transfer reactions within biological electron transfer chains.

Small Asymmetric Complexes of Electron Transfer Proteins as Fundamental Units for Energy Conversion

If electron transfer reactions from excited chlorophyll molecules are electrochemical oxidation reactions analogous to the type investigated at semiconductor electrodes, the photosynthetic electron acceptor system should behave like a polarized electrode--that is, it has to provide two main properties:

- a) The acceptor system has to have a solid-state-like structure, in which accepted electrons can spatially be transferred away from oxidized chlorophyll;
- b) it has to provide a gradient of electrochemical potential in which the electrons can be captured and transferred at a sufficiently high rate to prevent a reverse reaction.

These two essential conditions appear to be satisfactorily fulfilled, if it is assumed that the molecules of the photosynthetic electron transfer chain in chloroplasts which accept the electron from excited chlorophyll (Y, PQ, cyt.  $b_6$  for PS II; X, Fd for PS I; Z, Fd (?) in chromatophores) (see Fig. 3) are aggregated in a solid state-type complex within the membrane. An asymmetric complex of redox molecules of different redox potential, linked by bands and suitable ligands, which could decrease or remove activation barriers should actually provide solid-state-like electron transfer properties as well as an electrochemical potential gradient (which would be identical with a gradient of the redox potential of its constituents). In the photosynthetic electron acceptor system, only the very first electron acceptor molecules have to be bound tightly to meet the requirement of rapid electron capture from excited chlorophyll. Electron transfer to and between subsequent electron transfer

molecules can still be dominated by activation barriers.

Figure 4 shows schematically how electron transfer molecules of different redox potential can be thought of as forming a complex, in a membrane, which provides an electrochemical potential gradient and electronic properties adequate for the capture of electrons from excited levels of chlorophyll. As a part of the membrane it should behave like an electrode, at which excited chlorophyll could be oxidized in an electrochemical reaction.

In a limiting case, the electrode properties of the primary complex (A + B in Fig. 4) could be provided by a single, especially adapted electron transfer protein. It could capture electrons from the excitation level of a chlorophyll molecule with a suitable ligand at a lower redox potential and equilibrate the electrons spatially away from the chlorophyll to a redox center (e.g. metal ion) of a higher (positive) redox potential.

The application of electrochemical concepts suggests, further, that complexes of biological electron transfer proteins, which are oriented within an energy transducing membrane, able to oxidize a suitable electron donor (e.g. excited chlorophyll) of low redox potential and to reduce an electron acceptor (A) of higher redox potential at the opposite side of the membrane, may be looked upon as the basic energy converting units in biological electron transfer chains: From a physico-chemical and electrochemical point of view such complexes (see Fig. 5) constitute real, functioning molecular electrochemical redox elements, which are able to convert directly the free energy gain from the redox reaction  $\text{Chl}^* + \text{A} \longrightarrow \text{Chl}^+ + \text{A}^-$  into an electrical potential according to the relation:

$$\epsilon = - \frac{\Delta G}{nF} \quad (2)$$

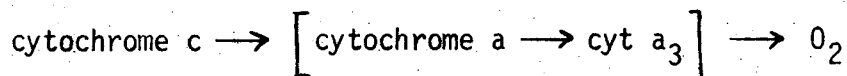
With respect to their functional properties, these molecular redox elements are identical to macroscopic electrochemical redox elements, in which electron transport from an anode to a cathode is warranted by an electric conductor. Within the proposed molecular redox elements, electron transfer from the anodic to the cathodic side of an energy transducing membrane is simply provided by the electron transfer properties of the electron carrier complex; and the possibility for the utilization of the electrochemical energy is warranted by the membrane itself. It provides a resistance for the ion transfer which will close the electrical circuit of the redox element (see Figs. 5 and 6).

The recognition that electron transfer across membrane-bound electron transfer complexes may be coupled directly with the liberation of electrochemical energy according to relation (2) considerably simplifies the concepts of energy conversion in energy transducing membranes (see below). An interesting conclusion can immediately be drawn, namely that for the production of electrochemical energy by the proposed redox elements, it is unimportant whether pure electron carriers or hydrogen carriers are involved in electron exchange reactions (see relation (2)). Consequently, the liberation or uptake of ions (protons) may be (at least in part) looked upon as secondary effects during energy conversion.

With respect to the important role that complexes of electron transfer proteins play in this redox element hypothesis, an example of the type of electron carrier complex, which is supposed to provide the structural properties for the function as molecular redox element, should be given: A series of complexes of different electron carriers, representing limited segments of the respiratory chain, have been described.<sup>3a,4</sup> Especially interesting is cytochrome oxidase, which is tightly

bound to mitochondrial structures, resists physical separation into subcomponents, but contains heme groups with distinct kinetic properties. The portion that is unreactive with HCN has been designated as cytochrome  $a_3$ , and the portion that reacts with cyanide as cytochrome  $a_3$ . The electron transfer sequence,

cytochrome oxidase



has been established (for reference see 3b) and it has been suggested that the functional unit of cytochrome oxidase consists of four a-heme units, two  $a_3$ -heme units plus six Cu atoms.<sup>5</sup> In the present model cytochrome oxidase, when properly arranged in the energy transducing membrane, upon accepting electrons from cytochrome c and donating them to oxygen, behaves as an electrochemical redox element and converts energy according to relation (2). The fact that cytochrome oxidase has been found to be the site of phosphorylation may support this hypothesis, especially as there is evidence that cytochrome oxidase can undergo redox reactions at both membrane surfaces.

The concept of a solid state-like electron transfer mechanism and a rapid equilibration of electrons within small complexes of biological electron carriers, which are prepositions for the operation of the redox elements, is especially supported by the investigations of R.J.P. Williams.<sup>6</sup>

Is the Redox Element Hypothesis Consistent with Experimental Data from Photosynthesis?

1) Construction of the photosynthetic membranes and the reaction centers

According to Figure 6, the electrochemical reaction of excited chlorophyll would occur at the interface between a membrane-bound oriented electron acceptor complex and an electrolyte. At the electrolyte side, bound or soluble electron donors should provide the regeneration of chlorophyll.

The electron acceptor complexes have to be constructed of constituents of different redox potential (which, in a limiting case, can also be built together into one electron transfer protein). The electron acceptor complex of photosystem II (PS II) can be thought of as being constructed of the carriers Y, PQ, cyt  $b_6$ ..., that of PS I of the unknown primary acceptor X and of membrane-bound ferredoxin. The existence of the latter within the membrane has been recently verified.<sup>7</sup> It has also been shown that this membrane-bound ferredoxin can be photo-reduced at liquid nitrogen temperature. In photosynthetic bacteria, an electron acceptor complex could be constructed of Z and possibly also of membrane-bound ferredoxin (in noncyclic electron transfer).

An aggregation of chlorophyll molecules in the reaction center would be unfavorable for the efficiency of electron capture, as charge migration across the aggregates would have to participate, which needs activation energy.<sup>8</sup> Consequently, only single or a few chlorophyll molecules should be present in the reaction center (visualized as square in Fig. 6), which will consist of a small primary electron acceptor complex with a few attached chlorophyll molecules and possibly a donor. Reaction centers of a molecular weight of 40,000 have been isolated.<sup>9</sup> The observation that they still function is consistent with the proposed conception of the electron capture system in photosynthesis, as are the X-ray studies by W. Kreutz.<sup>10</sup>

2) Electron capture, continuous electron flow, and quantum efficiency

The function of the electron acceptor complex, to capture electrons from excited chlorophyll molecules with high quantum efficiency and to generate a continuous electron flow, rests on the existence of an electrochemical potential gradient (gradient of redox potential). The time constant for the electron capture will be determined by the electronic structure and the solid state properties of the acceptor complex. It should be noted that an electron equilibration in an asymmetric complex of biological electron transfer molecules cannot be described on the basis of a semiconduction or hopping mechanism for electron transfer, since this would presuppose the existence of a periodicity of molecules or bonds. For the complicated electron acceptor complex in photosynthesis the only qualitative statement that can be made is that the potential energy  $U(x)$  of the electrons will decrease with increasing distance ( $x$ ) from the chlorophyll (see Fig. 4). In a simple approximation, the potential energy distribution  $U(x)$  can be looked upon as a superposition of the distribution of redox potential within the electron acceptor complex  $A(x)$  and a coulomb potential  $V(x)$ , arising from a charge separation (separation of the electron from chlorophyll):

$$U(x) = A(x) - e^2/\epsilon x$$

The probability  $W$  for finding the electron of the excited chlorophyll within the electron acceptor complex (volume  $V_{AC}$ ) can be calculated by applying the semi-quantum mechanical Wenzel-Kramers-Brillouin approximation:<sup>11</sup>

$$W = W_0 \int_{V_{AC}} w(x) dV = W_0 \int_{V_{AC}} |\psi(x)|^2 dV = W_0 \int_{V_{AC}} \frac{|c|^2 dV}{p(x)} \quad (3)$$

where  $p(x)$  describes the momentum of the electron in the  $x$  direction.

$$p(x) = \pm \left[ 2m_e \left( E - A(x) + \frac{e^2}{\epsilon \cdot x} \right) \right]^{\frac{1}{2}} \quad (4)$$

To obtain a high probability for capturing electrons from excited chlorophyll there are essentially two possibilities (see relations 3 and 4): One would be to provide a very big electron acceptor over which electrons can equilibrate. This possibility is obviously not utilized in photosynthesis, since very small reaction center preparations still function in electron capture. The second possibility, which is more likely for electron capture in photosynthesis, is a decrease of the total energy of the electron within the electron acceptor complex, which decreases the momentum of the electron  $p(x)$  and captures the electron in the potential dip of the electron acceptor (Fig. 4). This release of a small amount of energy will be the essential step for the electron capture, and can be stimulated by any interaction of the electron with the electron acceptor complex in which it loses momentum (e.g., by vibrational excitation). This interaction must have a relaxation time shorter than the lifetime of excited chlorophyll.

This type of electron capture from excited chlorophyll at an electron acceptor complex to which chlorophyll is bound will be very efficient and would not need activation energy. It would thus be compatible with experimental observations that, in photosynthesis, electron capture is effective down to 1 degree Kelvin.<sup>12</sup>

A continuous electron flow will be provided by the electrochemical gradient within the acceptor complex. In a calculation, however, it has to be considered that electric potentials will appear as a consequence



of electron transfer. In the mathematical treatment, the Poisson equation has to be combined with the Nernst-Planck equations, and suitable boundary conditions have to be chosen to account for electron exchange reactions at the surfaces. As a result, space charge limited electron transfer is to be expected at higher electron injection rates. Consequently, the internal resistance ( $R_e$ ) of the electron acceptor complex can be assumed to be ohmic only to a first approximation.

3) The production of a primary electric potential

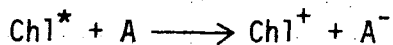
Within the discussed electrochemical concept, the delocalization of captured electrons in the electrochemical potential gradient of the electron acceptor complex is inevitably connected with the appearance of an electric potential at the reaction center. The appearance of the potential will occur with the time constant characteristic for the capture of electrons from excited chlorophyll--that is, it will have a time constant of at least  $10^{-8}$  sec.

H. T. Witt and associates,<sup>13</sup> and others,<sup>14</sup> were able to relate 518 nm absorption changes to the appearance of an electric field across the photosynthetic membrane. The rise time of this light-induced field was found to be shorter than  $2 \times 10^{-8}$  sec.

This result strongly supports the suggested view of the primary electron capture process in photosynthesis: firstly, it shows that a charge separation actually takes place; secondly, the fast rise time of the field can only be satisfactorily understood when some kind of solid state electron transfer is involved; and thirdly, an electron transfer which builds up an electric field will occur only when a driving force (an electrochemical gradient) is provided.

The observation of an electric field in the thylakoid membrane is strong justification for the initial statement that electrochemical kinetics has to be applied to electron transfer in photosynthesis.

The development of an electric field across the thylakoid membrane as a consequence of the electrochemical oxidation of excited chlorophyll is determined by the affinities of the electron donor (excited chlorophyll,  $\text{Chl}^*$ ) and a potential determining electron acceptor A (e.g., ferredoxin) within the membrane or the electrolyte at the opposite side according to the electron transfer reaction:



The maximum electromotive force which could be generated should be:

$$\epsilon = -\alpha \frac{\Delta G}{nF}$$

with  $\alpha = 1$  in the theoretical case of equilibrium and reversibility.

Because of irreversible components in the electron capture reaction, the proportionality factor will be smaller than unity. Introducing the activities of participating components and assuming that the activity of excited chlorophyll is proportional to the light intensity ( $I_L$ ) the following relation for the generated electromotive force can be obtained:

$$\epsilon = -\alpha \frac{\Delta G_0}{nF} - \alpha \frac{kT}{nF} \ln \frac{a_{\text{Chl}^+} a_{\text{A}^-}}{a_{\text{Chl}^*} a_{\text{A}}} = A + B \ln I_L \quad (I_L > 0) \quad (5)$$

Thus, the electric potential at the thylakoid membrane should show a logarithmic dependency on the light intensity. This relation should be valid, provided ion flux across the membrane is negligible and regeneration of the reaction center is not rate-limiting (open circuit condition).

4) Energy storage and energy conversion: membranes as parametric energy transformers

According to the present concept, the separation of the excited electron from the reaction center chlorophyll occurs by an electrochemical potential gradient within the electron acceptor complex. As a consequence, energy is primarily stored in the form of free energy of an electron behind the activation barrier, formed by the primary electron acceptor of lower redox potential, which prevents the electron from a reverse reaction with the oxidized chlorophyll (Fig. 4). Electrochemical considerations have led to the conclusion that a membrane-bound electron transfer complex, which is able to produce an anodic oxidation and a cathodic reduction of redox agents at opposite membrane sides, may be considered as a real electrochemical redox element which generates an electromotive force according to relation (2).

For a steady state electron transport from anode to cathode across the electron transfer complex (internal resistance  $R_e$ ), the electric circuit has to be closed by an ion flux across the membrane. In Fig. 6 a schematic arrangement of the proposed light-driven redox element within the thylakoid membrane is shown, as well as the positions of channels for ion transfer (resistance  $R_i$ ) and conversion of electrochemical energy (work resistance  $R_c$ ). Also the membrane capacity is depicted in Fig. 6b, which is an important parameter for the electrochemical dynamics of the redox element system.

For a steady state operation, a simple correlation between light induced electron transport ( $i$ ), the electromotive force ( $\epsilon$ ) of the redox element, the internal resistance ( $R_e$ ) of the electron transfer complex, the membrane potential ( $\psi$ ), the charge of the membrane and

membrane parameters, like resistance for ion transfer ( $R_i$ ) and work resistance for energy conversion ( $R_c$ ) is provided (Fig. 6b):

$$\psi = \epsilon - i R_e = i \frac{R_c \cdot R_i}{R_c + R_i} = \frac{q}{C} \quad (6)$$

The free energy of the electrons which have been captured in the electron acceptor complex is available in two forms:

- a) for reduction processes that generate molecules with low redox potential (e.g. NADPH) at the cathodic membrane surface, and
- b) as electrochemical energy of the redox element, which can again be converted into chemical energy, and would be available for phosphorylation.

In principle, the distribution of energy into these two forms can easily be controlled by providing potential determining electron acceptors with suitable redox potentials (see relation 5). In this connection it should, however, also be considered that an electric potential, in turn, will influence the value of redox potentials of membrane-bound electron transfer molecules. This is visualized in Figure 7, where the electron acceptor complex of PS I (assumed to be constructed of an unknown primary acceptor X and membrane-bound ferredoxin) is depicted in the free energy scheme. If the soluble ferredoxin at the cathodic membrane side is removed, electrons will accumulate at the membrane-bound ferredoxin, which will become the potential determining acceptor for the generation of an electrical potential ( $\Psi$ ). As a consequence of this, according to relations (1) and (2), the redox potential of membrane-bound ferredoxin will decrease and approach the redox potential of the primary acceptor X. Photosystem I should therefore be able to reduce soluble electron

acceptors with redox potentials up to approximately -600 mV. (This is in accordance with experimental data, obtained by B. Kok and others, with methylviologens.<sup>15)</sup>)

The rate at which electrochemical energy is being turned over at the membrane can be obtained by multiplying the terms in equation (6) by the flux of transferred currents (electron current  $i$ , ion current  $i_i$ ,  $i_c$ ). In the resulting equation the consideration of the time-dependent term  $\frac{d}{dt} (\frac{1}{2} C\psi^2)$  is essential. It considers a possible non-steady-state way of converting energy, namely by changing the amount of capacitive energy which is stored in the energy-transducing membrane.

$$\epsilon_i - i^2 R_e = i_i^2 \frac{(R_c R_i)}{(R_c + R_i)} + \frac{d}{dt} (\frac{1}{2} C\psi^2) \quad (7)$$

The first term in this equation is the energy which is produced by the light-driven redox element (Fig. 6). The second term describes the energy which is dissipated in transferring electrons through the electron transfer chain. The third term describes the energy required to pump ions across the membrane and to drive endothermic electrochemical reactions in suitable membrane channels. The last term is especially interesting in that it shows that capacitive energy can be stored and released in the membrane by a change of membrane parameters like geometry ( $g$ ), dielectric constant ( $\epsilon$ ) or membrane potential ( $\psi$ ):

$$\Delta G_c = \frac{dW}{dt} = \frac{d}{dt} (\frac{1}{2} C (\epsilon, g, \psi) \psi^2) = \frac{\partial W}{\partial \epsilon} \frac{d\epsilon}{dt} + \frac{\partial W}{\partial g} \frac{dg}{dt} + \frac{\partial W}{\partial \psi} \frac{d\psi}{dt}$$

A rapid structural change within the energy transducing membrane (change of  $g$ ,  $\epsilon$ ) could easily be stimulated, after a certain (critical) membrane

potential ( $\psi_c$ ) has developed. If the change decreased the membrane capacity  $C(g, \epsilon, \psi)$  this would trigger the release of a corresponding amount of capacitive energy within the membrane (according to relation 7). Provided there is a chemical reaction to consume the capacitive surplus energy with a time constant which is short compared with the time constant for the discharging of the membrane capacity by reverse of electron and ion transfer ( $\frac{1}{k} \ll RC$ ), capacitive electrochemical energy would be converted into chemical energy.

In order to decide whether a steady state or a non-steady state (periodic) mechanism of energy coupling is involved in energy conversion at energy-transducing membranes, some general aspects of electrochemical coupling should be considered. In energy conversion mechanisms, in which free energy of molecules ( $\Delta G$ ) is converted in a steady state manner into electrochemical energy ( $-nF\psi$ ) and this again into another energy form (chemical energy, mechanical energy), it is important to distinguish between efficiency of energy conversion (= % of energy converted in the overall process) and power output (energy converted per unit time). The electrochemical energy of a fuel cell or a battery theoretically can be converted with almost 100% efficiency when the energy is drawn only at an infinitesimally small time rate (this means low power output, low ion current over very high work resistance  $R_c, R_i$ ) (see Fig. 6b). If it is desired, however, to get as much electrochemical energy converted as possible per time (maximal power output), the external work resistance has to be adjusted (matched) to the internal ( $R_{ext} = R_{int}; R_i R_c / (R_i + R_c) = R_e$  in Fig. 6b), which can be shown by simple calculations (differentiation of equation 7 without last term).

In this case, half of the energy is dissipated at the internal resistance of the potential generating source. The efficiency of energy conversion at the external work resistance is only 50%; the power generated, however, reaches its maximum, also 50%.

There is, however, an essential difference between fuel cells and batteries, on the one hand, and energy-transducing membranes which produce electrochemical energy as an intermediate, on the other. If the electric wires connecting anode and cathode of a battery or a fuel cell are disconnected, the electrochemical energy remains stored in the element. It is therefore possible to convert energy at a slow rate, but with high efficiency. On the other hand, the electrochemical energy which is stored at an energy-transducing membrane is rather rapidly discharged according to an exponential law:

$$W = \frac{1}{2} C\psi^2(t) = \frac{1}{2} C\psi_0^2 e^{-\frac{2t}{RC}}$$

R,C = resistance, capacity of membrane

because the energy-transducing membrane is sufficiently permeable for ions. The most effective way, therefore, to convert electrochemical energy at an energy-transducing membrane is to do it at a high rate, in order to compete effectively with the dissipation of electrochemical energy.

This conclusion can be clarified by examining the case of noncyclic electron transfer in photosynthesis: Light-induced electron transfer and reduction of NADP will proceed regardless of whether photophosphorylation occurs or whether it is inhibited by lack of ADP. As electrochemical energy cannot be continuously accumulated and stored, an energy conversion mechanism, which utilizes electrochemical energy for

phosphorylation, should be functioning in such a way that it provides the highest possible power output and utilizes the continually generated electrochemical energy at the highest possible rate.

A steady state mechanism of electrochemical coupling appears therefore to be unfavorable for energy conversion during phosphorylation. In oxidative phosphorylation 50% of the energy available from the electron transfer reactions reappears in the form of pure chemical energy of ATP,<sup>3c</sup> even though some parts of the electron transfer chain do not contribute to phosphorylation. Besides, a considerable amount of energy would have to be supplied for mechanochemical and osmotic energy.<sup>3c</sup>

It is important to note here that the chemiosmotic mechanism<sup>16</sup> (which assumes that electron transfer by asymmetric proton translocation is generating a proton-motive force which drives a proton-current over a reversible ATPase for ATP formation) has to be looked upon as a special case of such a steady state electrochemical energy conversion mechanism. (P. Mitchell<sup>17</sup> has compared this mechanism with that of a fuel cell based on proton transfer.) This chemiosmotic fuel cell, however, would have the unfavorable attribute of discharging itself rather rapidly and of having only a limited capacity to store energy, and would have to be operated near maximal power output conditions. Under these conditions, the chemiosmotic mechanism could only explain energy conversion with 50% efficiency, and would therefore be unable to explain phosphorylation.

The limitations of a steady state mechanism for conversion of electrochemical energy do not apply to a non-steady state periodic mechanism (as indicated by the last term in relation 7) of conversion



of electrochemical energy into chemical energy of ATP. Such a time-dependent, periodic mechanism of energy conversion is extremely interesting in many ways. Generally, the principle of the suggested oscillating mechanism of energy conversion (which involves periodic changes of the membrane capacity) can be summarized in the following way: When an energy absorbing system (in the present case, chemical reaction leading to ATP) is suitably coupled to an energy storage element (charged membrane) whose value is made to vary in a proper way (conformation changes leading to changes in membrane capacity), energy may be extracted from the source which drives the energy storage element (electrochemical redox elements) and transferred to the energy absorbing system (chemical reactions leading to chemical products)(see Fig. 8).

Energy transducing or amplifying systems which are based on such a principle are called variable parameter, or parametric energy transformer, or amplifier. The principle of operation of these parametric energy transducers was first described by Lord Rayleigh,<sup>18</sup> who used a mechanical model. Their operation can easily be visualized with the help of the simple example of a child on a swing. By lifting his weight up and down with a period two times that of the swing, thus varying the effective length of the ropes (parameter), it pumps energy to increase the amplitude of the swing. In more recent times, the principle has been successfully applied for electronic and microwave circuits.<sup>19-21</sup> Capacitances, inductors, ferrimagnetic materials, semiconductor devices, ferro-electric materials and electron beams (kinetic energy) have been applied or suggested as variable energy

storage elements. In these energy storing elements a parameter is usually not periodically changed by external interference (like in the vibrating condenser amplifier). Rather, the fact is utilized that parameters (e.g., the capacity of a diode) may not be really constants, but may depend on the amount of stored energy. In these systems the change of the parameter is already guaranteed by the nonlinear behavior of the elements.

The application of the principle of parametric energy coupling to an electrochemical system like an energy transducing membrane is a straightforward matter. To determine the exact mode of its operation in energy transducing membranes, however, careful theoretical and experimental investigations will be needed. It is important to note that both the energy which is utilized for performing the conformation changes (parametric changes) and the pure capacitive energy can be supplied from the same electrochemical source (redox elements) in the energy transducing membrane, and that this dual function would simply result from a nonlinear dependence of the membrane capacity on the stored energy.

The most interesting aspect of the electrochemical parametric energy transformation mechanism is that it is in agreement with much important experimental data:

- 1) Conformation changes and performance of mechanical work during energy cycles, which have been reported for mitochondria,<sup>22,23</sup> can be looked upon as periodic changes of the membrane capacity, which trigger the conversion of capacitive energy into chemical energy according to the principle of a parametric energy transformer.

- 2) The parametric mechanism of energy conversion can be reversed (ATP → conformation changes → electrochemical energy); consequently, ATP should be able to trigger conformation changes.

The parametric energy transformation mechanism makes a bridge between concepts of electrochemistry and membrane structure dynamics, provides an energetic explanation for its interrelation, and a phenomenological basis for mathematical calculations. The electrochemical parametric energy conversion mechanism is therefore compatible with the conformation change hypothesis,<sup>22,23</sup> in that conformation changes are important factors in energy conversion. There are, however, essential differences: The chemical energy of electron transfer reactions is not stored primarily in the energy of conformation change, as the conformation change hypothesis assumes, but energy is stored as electrochemical energy of a charged membrane (which can undergo some conformation changes as a result of charging), and conformation changes trigger conversion of electrochemical energy. Therefore, conversion of energy should be inhibited, if charging of the membrane is sufficiently suppressed, for example by leaks in the membrane, which act as short circuits.

Thus, according to the considerations presented here, the following mechanism leading to ATP formation is the most likely within the redox element hypothesis (see Figs. 6, 8, and especially Fig. 9, in which the variation of capacitive energy with changes in the membrane potential and membrane conformation during the energy conversion cycle is depicted). Electrochemical reactions at electron transfer complexes lead to the development of an electric potential at the energy transducing membrane

(according to relations 2 and 3) and to a charging of the membrane capacity ( $R_e$  can be very small). The charging may involve structural changes of the membrane:  $C = C(g, \epsilon, \psi)$  and leads to an energized membrane (path 1  $\rightarrow$  2 in Fig. 9). When the potential is sufficiently high, a sudden structural change (change of  $\epsilon, g$ ) within the membrane (transition to energized twisted configuration) (2  $\rightarrow$  3 in Fig. 9) will decrease the membrane capacity and trigger the release of capacitive energy as chemical energy  $\Delta G_c$  for (ATP) (3  $\rightarrow$  4); then the original membrane capacity is reestablished (4  $\rightarrow$  1) and a recharging can occur. For energetic reasons (effective charging of the membrane capacity to provide the possibility of parametric energy conversion), it has to be assumed that the membrane resistances  $R_c$  and  $R_i$  are relatively high. Ion transfer across the membrane should predominantly occur as a result of the variation of the membrane capacity (conformation change) and may be considered as composed of a capacitive ion transfer which recharges the membrane ( $i_c = \frac{dq}{dt}$ ) and an ion transfer which results from the chemical reactions leading to ATP.

The reverse of the capacitive energy conversion mechanism is indicated in Figure 9. The presence of an energy rich chemical agent (ATP) will trigger an increase of the membrane capacity (1  $\rightarrow$  2'). To gain the energy, which is needed as capacitive energy, at the membrane potential  $\psi_1$ , chemical energy of ATP is utilized (2'  $\rightarrow$  3') and, after the original membrane capacity is reestablished (3'  $\rightarrow$  2), the membrane is in an unstable energized state, and is discharged by driving ion and electron transfer in the reverse direction (2  $\rightarrow$  1).

The amount of capacity change and thus of conformation change involved in the parametric energy conversion mechanism is difficult to

predict, as it will depend on the special mode of operation and the characteristic properties of the membrane. Simple estimates, which compare the capacitive energy of a charged membrane unit with the energy of an ATP molecule, show that once a membrane is charged, periodic capacity changes of as low as a few percent and less would be sufficient to maintain phosphorylation. It is interesting to note that there is a lag in phosphorylation to be expected after the onset and termination of electron transfer activity, which will be determined by the time required to charge or discharge the energy transducing membrane. Speculations on the chemical reaction which utilizes the surplus capacitive energy of the charged membrane in its energized (twisted) conformation (3 in Fig. 9) and leads to ATP synthesis, are beyond the scope of these investigations.

The proposed parametric energy coupling mechanism could give an energetic and dynamic explanation of the complicated interrelations of electrochemical, structural, mechanical and chemical processes in biological membranes. Theoretical investigations of this mechanism are needed, for which the mathematical formalism that has been developed for electronic application<sup>24</sup> will be useful.

##### 5) "So-called" solid state effects

A series of solid state effects have been reported by investigators of photosynthesis, and any complete model of photosynthetic processes must account for them. These effects, mostly measured with dried chloroplasts, comprise photoconductivity,<sup>12,25,26</sup> high light-induced polarizability,<sup>12</sup> thermoconductivity,<sup>27</sup> thermoluminescence,<sup>27</sup> microwave absorption,<sup>28,29</sup> and microwave Hall effect.<sup>29,30</sup>

Hypotheses on the existence of semiconductor properties in chloroplasts and their possible role in photosynthesis have been put forward in the last three decades by a number of investigators.<sup>31-33</sup> It has been assumed by most of these authors that chlorophyll aggregates in the chloroplast are responsible for solid state effects, and numerous model experiments with layers of chlorophyll have been made to test this idea.<sup>34-37</sup>

According to our electrochemical considerations, a mechanism in which hole or electron migration across a layer of aggregated chlorophyll participates in the energy conversion process of photosynthesis would restrict efficiency. Consequently, there is no reason to assume that semiconductor properties of chlorophyll in vivo are essential processes in photosynthesis.

Moreover, the primary proposal that electrons can easily be delocalized within the well organized electron acceptor complex (or holes in a donor complex), is completely adequate to explain the reported solid state effects. Electrons (or holes) injected by reaction center chlorophyll into such electron carrier complexes, would be mobile there and could be accelerated by externally applied electric fields. Consequently, illumination of the chloroplast will produce chlorophyll sensitized photoconduction, as electrons can be transferred across the thylakoid membrane (provided ion conduction is not rate limiting) and microwaves should be absorbed by the semifree electrons. These effects should have a very short rise time of faster than  $10^{-8}$  sec corresponding to the rapid electron capture by the electron acceptor complex. The decay time should be slow ( $10^{-4}$  sec to several sec), as the electrons

are captured in the complex and their consumption will be determined by reactions in the electron transfer chain, which will release them from the membrane, or by reverse reactions by thermal activation (thermoluminescence, delayed light).

6) Delayed light emission, electron transport and electric potentials in photosynthesis

Electrons, which have been injected into the electron transfer complex, and the remaining hole (oxidized chlorophyll or an oxidized electron donor), have a chance to recombine in a reverse reaction and to produce emission of delayed light (Fig. 4). In describing the rate constant for the reverse reaction of an electron, it has to be considered that, depending on the polarity, an electric potential will increase or decrease the necessary activation energy. This is visualized in Figure 10 for a potential that decreases activation energy.

When  $\lambda(x) \cdot \psi$  is the electric potential at the position of a certain electron carrier within the membrane (point  $x$ ) from where the thermal activation should occur,  $n(x)$  the probability of finding electrons there, and  $p(x)$  the probability that chlorophyll is oxidized, the intensity of delayed light emission (from this carrier) may be described as:

$$I_{DL}(x) = k(x) n(x) p(x)$$

with

$$k(x) = k_0 \exp \left[ -\frac{1}{kT} (\Delta G_A(x) - \lambda(x) \cdot F \cdot \psi) \right] \quad (8)$$

where  $\Delta G_A$  is the free energy of activation without potential.

With relations (5) and (6), consequently, the intensity of delayed light emission from a certain electron carrier in a steady state condition (point  $x$  in complex) will be:

$$I_{DL}(x) = p(x) n(x) k_0 \exp \left[ -\frac{1}{kT} (\Delta G_A(x) - \lambda(x) F(A+B \ln I_L - iR_e)) \right] \quad (9)$$

It is therefore dependent on the carrier from which the electron is activated ( $G_A(x)$ ,  $\lambda(x)$ ), on the probability of finding an electron there  $n(x)$ , the light intensity ( $I_L$ ) and the electron flux ( $i$ ) within the electron transfer chain, which is dependent on membrane properties ( $R_i, R_c$ ) according to relation (6). The delayed light emission (according to relation 9) will increase when the electron transfer ( $i$ ) is decreased by inhibition, or when phosphorylation or ion transfer is inhibited ( $R_c, R_i \rightarrow \infty$ ). On the other hand, the emission of delayed light should decrease when the latter activities are enhanced (addition of ADP or use of detergents).

The rise and decay of delayed light emission should go parallel to the development and decay of the potential at the electron acceptor complex. The generation of the electric potential within the complex will be initially determined by the reaction constant of the electron capture process, which should be faster than  $10^{-8}$  sec, and subsequently, be influenced the electrochemical activity which leads to a charging of the membrane and to a steady state situation.

The decay of delayed light emission after termination of illumination will be determined by the discharge of the membrane capacity, which may be assumed to follow an exponential law:

$$\psi = \psi_L \exp \left( -\frac{t}{RC} \right) = \psi_L \left( 1 - \frac{t}{RC} + \left( \frac{t}{RC} \right)^2 - \dots \right)$$

If this decay function of the membrane potential is inserted in relation (8), the total decay characteristic of the delayed light is obtained.



The first term of the series yields the steady state relation (9), the second describes the decay of delayed light immediately after stopping illumination ( $t \ll RC$ ;  $n(x,t), p(x,t) \approx \text{const}$ ):

$$I_{DL}(x,t) = I_{DL}(x,t=0) \exp \left[ - \frac{1}{kT} (\lambda(x) \cdot F \cdot (A+B \ln I_L - i \cdot R_e) \frac{t}{RC} \right] \quad (10)$$

It follows a first order reaction which, interestingly, has a mean relaxation time that decreases with increasing intensity of preillumination ( $I_L$ ).

Very recently, kinetic experiments on delayed light emission from Chlorella pyrenoidosa have been performed by C. Bonaventura and M. Kindergan.<sup>38</sup> These investigators resolved a fast exponentially decaying component, which actually showed a relaxation time that decreased with increasing light intensity ( $W = 0.1 \text{ mW/cm}^2$  :  $t_{1/2} = 9.57 \text{ ms}$ ;  $W = 0.9 \text{ mW/cm}^2$  :  $t_{1/2} = 5.74 \text{ ms}$ ;  $W = 12 \text{ mW/cm}^2$  :  $t_{1/2} = 3.3 \text{ ms}$ ). A similar light intensity dependency of the fast exponential decay of delayed light has also been found with chloroplasts recently.<sup>39</sup> These experimental results are considered as an essential support for the applicability of electrochemical concepts of photosynthesis, as they explain the kinetics without the need for special assumptions (see relation 10).

Artificial ion gradients produced at photosynthetic membranes can generate diffusion potentials  $n(t)$  which have to be added to the membrane potential ( $\psi$ ) in equations (6), (7) and (8):  $\psi \rightarrow \psi \pm n(t)$ . In this expanded form, these relations also describe the ion gradient effects on electron transfer, ion transfer, energy conversion<sup>40</sup> and delayed light emission.<sup>41-46</sup> Qualitatively, they predict an increase of delayed light emission, when the action of the diffusion potential is parallel to the photopotential, a decrease when it is antiparallel. Furthermore,

they show that a diffusion potential (e.g. proton gradient potential) can replace the photopotential in the discussed process of energy conversion which leads to phosphorylation, and in the generation of ion transfer.

Although quantitative verification of the derived relations has not yet been possible, because of the lack of well controlled experiments, it seems that they are able to describe the very complicated kinetics of delayed light emission, the influence that various parameters exert, and the effect of artificial ion gradients on electron transfer, ion transfer, phosphorylation, and delayed light emission in a consistent and qualitatively correct way.

The results which have been derived for delayed light emission support and are consistent with original ideas by Crofts,<sup>47</sup> Amesz and Kraan,<sup>46</sup> Fleischmann,<sup>48</sup> and Kraan and others,<sup>49</sup> who have proposed an influence of membrane potentials on delayed light emission.

7) The coupling of electron transport with ion translocation and phosphorylation

For the operation of the proposed redox elements (Fig. 6) and thus for energy conversion, both electron transfer and ion transfer are essential. However, the free energy of electrons (photoactivated in photosynthesis) actually provides the driving force for all energy conversion activities, whereas ion reactions will have a controlling influence, where they become rate-limiting for the entire process. Once excited chlorophyll can be oxidized at the anode and a suitable agent of higher redox potential reduced at the cathode and electrolytic charge transfer can be maintained from cathode to anode across the membrane, the redox element will convert energy, regardless of what ions are involved. Consequently, from the appearance of a light induced pH gradient in chloroplasts, it cannot be concluded that a proton-motive force is an intermediate for phosphorylation (chemiosmotic

hypothesis<sup>16</sup>). It can only be concluded that protons are consumed or liberated during the special redox reactions involved in photosynthesis, and that protons participate in maintaining the ion transfer across the thylakoid membrane. If redox agents in photosynthesis, which involve proton exchange, would be replaced by similar redox agents which do not, energy conversion and ion transfer should still occur according to relations (2), (6) and (7).

The development of ion gradients is an inevitable consequence of electrochemical activity at the redox elements, as well as of specific ion transfer across the membrane. It may be regarded as a secondary effect which arises because of rate-limited diffusion processes and specific ion permeabilities across the membrane. Ion gradients tend to decrease the light-induced electromotive force ( $\epsilon$ ) of the redox element by their concentration-polarization potential ( $\psi_c$ ). Another type of polarization potential ( $\psi_p$ ) which is also directed against the original electromotive force ( $\epsilon$ ) can arise as a consequence of rate-limiting chemical reactions in which potential determining redox reactions at the membrane are involved. For quantitative calculations of the electrolytic ion transfer across the membrane and the arising concentration gradients, the electrolytic and membrane-specific transference numbers of participating ions have to be determined. Also, corrections for water transference effects in ion hydration shells have to be made which, in addition to osmotic effects, can contribute to a swelling or shrinking of membrane enclosed volumes. The presented calculations and qualitative considerations led to the conclusion that not a proton gradient (chemiosmotic hypothesis<sup>16</sup>), but the electrochemical energy of molecular redox elements, is the primary energy-rich

intermediate for energy conversion. The electrochemical activity of the redox elements then charges the energy transducing membrane in which chemical energy is produced by a parametric energy conversion mechanism. These results are applicable for both photosynthesis and oxidative phosphorylation.

The concept which has been derived here appears to be compatible with experimental data, which also support Mitchell's chemiosmotic hypothesis and are, at the same time, compatible with results that apparently do not: Among these are the observation of light-induced phosphorylation in the absence of an adequate pH gradient,<sup>50-52</sup> difficulties in explaining the stoichiometry of ion transfer,<sup>53-55</sup> phosphorylation activity in sonicated chloroplasts,<sup>50-52,56</sup> structural changes within energy transducing membranes,<sup>22,23</sup> which support the conformation change hypothesis of phosphorylation<sup>22,23</sup> and differences in kinetic parameters between proton uptake and phosphorylation (e.g., pH dependency<sup>57</sup>).

Within the redox element conception, ion transfer and accumulation is determined by the kind of redox reactions involved, as well as by the specific membrane permeabilities for ions. Predictions are only possible with a detailed knowledge of the corresponding parameters. Furthermore, a proton gradient does not have to appear as an intermediate step for phosphorylation, and membrane fragments will still be able to synthesize ATP, provided the resistance for the bypass of ion currents is high enough to allow sufficient charging of the membrane fragments. Proton gradients (provided they arise as a result of electrochemical activity) and the hypothetical high energetic

intermediate (X) leading to phosphorylation, should only be looked upon as the result of the same basic phenomenon--the operation of the redox elements. They will consequently show some similarities in response. However, the ultimate high energetic intermediate is most likely the capacitive surplus energy of the charged energy transducing membrane (which is converted into a chemical product), and there is no reason to expect a strict parallelism in the effect of agents on pH gradient formation and ATP formation. It might be interesting to note that the electrochemical energy of redox elements could be used to produce a proton gradient in a well determined way. This could be accomplished with the help of a membrane which is asymmetrical in its permeability for protons. Such a membrane would have properties which are equivalent to that proposed in the chemiosmotic hypothesis. At the present stage of experimental evidence, however, there is no need for such an assumption.

8) Remarks on oxygen evolution

Electrochemical kinetics has not only to be applied to electron transfer in biological electron transfer chains, but also to hole transfer, when membrane-bound carriers are involved. An example, where this seems to be necessary, are the reactions which lead to oxygen evolution in photosynthesis. In analogy to the mechanism of electron capture, holes can also be rapidly captured from excited chlorophyll by a suitable hole acceptor complex ( $Mn^{++}$ -protein complex?). Such a complex could capture holes in subsequent steps and eventually oxidize two molecules of water in the course of an energetically favorable electrochemical reaction in which four holes are involved. Hole

capture and reverse reactions of holes would be determined by dynamics similar to that of electron reactions. In particular, it should be expected that with every captured hole the probability of a reverse reaction is increased until the hole acceptor system can be discharged during oxygen evolution.

Application to oxidative phosphorylation: The concepts which have been developed here mainly for photosynthetic systems can, without difficulties, also be applied to oxidative phosphorylation with the only difference that the primary electron donor will be a ground state molecule of low redox potential and that the arrangement of electron transfer molecules to complexes with redox element properties will be one particular to the respiratory chain. An important advantage arises from the fact that it is only of subordinate significance for energy conversion whether there are electron carrier or hydrogen carrier involved in electrochemical reactions. There is consequently no need to postulate the existence of ubiquinone between cytochrome b and cytochrome c<sub>1</sub> of the respiratory chain, as it has been done to enable the application of the chemiosmotic concept<sup>16</sup> to oxidative phosphorylation.

#### Summary

Electrochemical experiments with excited chlorophyll have suggested the possibility that electron capture in photosynthesis may be an electrochemical oxidation of chlorophyll at a suitable electron acceptor complex with electrode properties. This stimulated an attempt to apply electrochemical kinetics to biological electron transfer reactions, which led

to the conclusion that small complexes of membrane-bound electron transfer molecules which oxidize a suitable donor on one membrane side and reduce an acceptor molecule at the opposite side may be looked upon as real electrochemical redox elements. An analysis of this concept and application of electrochemical calculations showed that it:

- 1) is compatible with the present knowledge about the organization of biological electron transfer molecules, the structure of energy transducing membranes, and of photosynthetic reaction centers.
- 2) would explain high quantum efficiency and effectivity of the electron capture mechanism down to very low temperature.
- 3) can explain the appearance of a fast, light-induced field across the thylakoid membrane.
- 4) is consistent with reported solid state effects which are sensitized by chlorophyll but originate within the electron acceptor (or donor) system.
- 5) can explain the dynamics of delayed light emission and provides a basis for the understanding and calculation of the influence of various parameters upon luminescence and electron capture.
- 6) is able to explain ion transfer and the development of ion gradients across the energy transducing membrane.
- 7) is able to explain the conversion of photochemical energy into chemical energy and suggests a time dependent parametric energy transduction mechanism as the most efficient way for conversion of electrochemical energy (of redox

elements) into chemical energy (of ATP).

8) is applicable in the same way to oxidative phosphorylation.

It contradicts the chemiosmotic hypothesis,<sup>16</sup> in that it states that:

- a) the basic structural and functional unit for the conversion of free energy of electrons into electrochemical energy is that of a redox element of the proposed type.
- b) proton gradients should only appear when the special electrochemical redox reactions at the proposed redox elements involve proton exchange, when protons are used to maintain electrolytic transfer across the membrane, or when the gradient is generated during phosphorylation.
- c) there is consequently no need for postulating special arrangements of hydrogen carriers to provide asymmetric proton translocation across energy transducing membranes.
- d) the energy conversion mechanism of the chemiosmotic hypothesis (proton current over reversible ATPase across membrane) gives a smaller power output than the proposed capacitive energy conversion mechanism (parametric energy conversion principle) and suggests that it may be too inefficient to explain energy conversion in respiration.

The proposed concept of parametric energy conversion is compatible with the conformation change hypothesis of energy conversion<sup>22,23</sup> in that it considers active conformation changes as essential events during the energy conversion cycle. It differs from it, however, as conformation changes are not primary reactions which store the chemical



energy of redox reactions. Rather, their main function is to trigger conversion of electrochemical energy by generating the variation of a parameter (capacity), which is the essential basis for parametric energy coupling.

The concept that has been developed here is only one of several possible mechanisms which, in principle, could explain interrelationships between electron transfer, membrane potential, and oxidative phosphorylation in energy transducing membranes (see E. A. Liberman and V. P. Skulachev<sup>58</sup>). Its justification, however, might be given by the following points:

- a) It is a simple one.
- b) It is able to describe primary reactions (e.g., photosynthetic electron capture) and secondary reactions (e.g., energy conversion) as well.
- c) The dynamics of reactions can be calculated in a straightforward way by applying concepts of electrochemical kinetics.
- d) It leads to a principle of energy coupling that has not yet been suggested for biological membranes.

The author is well aware of the fact that the proposed concept could be considerably refined and elaborated and will need further elaboration and refinement. This, however, will only be significant when the basic principles that have been suggested can be affirmed by detailed survey of the enormous amount of existing experimental data and by new experiments. This is far beyond the possibility of a single investigator. Therefore, a main aim of this paper has been to challenge and to stimulate criticism.

### Acknowledgements

The author wishes to thank the "Deutsche Forschungsgemeinschaft" for the grant of a fellowship. He is indebted to Drs. John M. Olson, Kenneth Sauer and Charles Weiss, Jr. for criticism of the rough copy of this work, to Drs. Roberto Bogomolni, Günter Petermann and Melvin Klein for stimulating discussions and encouragement, and to Dr. Melvin Calvin for his interest. The author is grateful to Dr. Robert M. Macnab for correcting the manuscript. This work was supported, in part, by the U.S. Atomic Energy Commission.

### References

1. K. Seifert and H. Witt, in "Progress in Photosynthesis Research," II, Helmut Metzner (ed. & Publ.), Tübingen, 1969, p. 750.
2. H. Tributsch and M. Calvin, Photochem. Photobiol., in press (1971).
3. Albert L. Lehninger, "The Mitochondrion," Benjamin Press, Inc., New York, 1965, a) p. 79, b) p. 76, c) p. 114.
4. D. E. Green and H. Baum (eds.), in "Energy and the Mitochondrion," Academic Press, New York and London, 1970, p. 77.
5. Q. H. Gibson and C. Greenswood, Biochem. J., 86 (1963) 541.
6. R. J. P. Williams, in "Current Topics in Bioenergetics," D. Rao Sanadi (ed.), Academic Press, New York and London, 1969, p. 79.
7. R. Malkin and Alan J. Bearden, Proc. Natl. Acad. Sci. U.S.A. 68 (1971) 16.
8. R. C. Nelson, Photochem. Photobiol. 8 (1968) 441.
9. G. Feher, Photochem. Photobiol., in press (1971).

10. W. Kreuzt, in "Progress in Photosynthesis Research," I, Helmut Metzner (ed. & Publ.), Tübingen, 1964, p. 91.
11. D. I. Blokhintsev, in "Principles of Quantum Mechanics," Sven Bjorklund (ed.), The George Washington University, Boston, 1964, p. 135.
12. W. Arnold and R. K. Clayton, Proc. Natl. Acad. Sci. U.S. 46 (1960) 769.
13. Ch. Wolf, H. Buchwald, H. Ruppel, and H. T. Witt, Naturwiss. 54 (1967) 487; H. T. Witt, in "Fast Reactions and Primary Processes in Chemical Kinetics," S. Claesson (ed.), Nobel Symposium V, Almqvist & Virsell, Stockholm Intersc. Publ., New York, London, Sydney, 1967, p. 261; in "Progress in Photosynthesis Research," II, Helmut Metzner (ed. & Publ.), Tübingen, 1969.
14. J. B. Jackson and A. R. Crofts, Eur. J. Biochem., 18 (1971) 120.
15. B. Kok, H. J. Rurainski, and O. V. H. Owens, Biochim. Biophys. Acta, 109 (1965) 347.
16. P. Mitchell, Nature 191 (1961) 144; Biochem. J. 81 (1961) 24; Biol. Rev. Cambridge Phil. Soc. 41 (1966) 445.
17. P. Mitchell, Fed. Proc. 26 (1967) 1370.
18. Lord Rayleigh, Phil. Mag. S.5 (1883).
19. V. D. Landon, RCA Rev. 10 (1949) 387.
20. H. Suhl, J. Appl. Phys. 28 (1957) 1225.
21. H. Heffner and G. Wade, J. Appl. Phys. 29 (1958) 1321.
22. P. D. Boyer, in "Oxidases and Related Redox Systems," T. E. King, H. S. Mason, and M. Morrison (eds.), Vol. 2, John Wiley and Sons, Inc., New York, 1964.

23. a) D. E. Green, G. Asai, R. A. Harris, and G. T. Penniston, Arch. Biochem. Biophys. 125 (1968) 684; b) D. E. Green and H. Baum, in "Energy and the Mitochondrion," Academic Press, New York and London, 1970.
24. K. N. Chang, "Parametric and Tunnel Diodes," Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 1964.
25. W. Arnold and H. K. Macley, Brookhaven Symp. in Biol. II, 1959.
26. S. Ichimura, Biophys. J., 1 (1960) 99.
27. W. Arnold and H. K. Sherwood, Proc. Natl. Acad. Sci. U.S., 43 (1957) 105.
28. L. A. Blymenfel'd, D. I. Kafaliyev, V. A. Livshits, I. S. Solov'yev, and A. G. Cheherikov, Reports of Academy of Sciences, U.S.S.R. Vol. 193, No. 3, 1970.
29. R. Bogomolni and M. P. Klein, reported on "International Conference on the Photosynthetic Unit," Gatlinburg, Tennessee, 1970.
30. D. D. Eley and R. Pethig, Bioenergetics, 2 (1971) 51.
31. E. Katz, in "Photosynthesis in Plants," W. Loomis and G. Franck (eds.), Iowa State College Press, Ames, 1949, p. 291.
32. M. Calvin, J. Theoret. Biol., 2 (1961) 258.
33. R. L. Strehler and W. Arnold, J. Gen. Physiol., 34 (1951) 80.
34. R. C. Nelson, J. Chem. Phys., 27 (1957) 864.
35. W. Arnold and E. Macley, in "Structure and Function of the Photosynthetic Apparatus," Inosct. Lit., Moscow, 1962, p. 9.
36. D. D. Eley and S. Smart, Biochim. Biophys. Acta, 102 (1965) 379.
37. K. T. Mc Cree, Biochim. Biophys. Acta, 102 (1965) 90 and 96.
38. Celia Bonaventura and M. Kindergan, Biochim. Biophys. Acta, in press.

39. Ora Canaani, M.S. Thesis, Weizmann Institute, Israel, 1970, personal communication.
40. E. G. Uribe and A. T. Jagendorf, *Plant Physiol.*, 42 (1967) 697, 706.
41. B. C. Mayne and R. K. Clayton, *Proc. Natl. Acad. Sci. U.S.*, 55 (1966) 494.
42. B. C. Mayne, *Photochem. Photobiol.*, 8 (1968) 107.
43. C. D. Miles and A. T. Jagendorf, *Arch. Biochem. Biophys.*, 123 (1969) 711.
44. C. D. Miles and A. T. Jagendorf, *Biochemistry*, 9 (1970) 429.
45. J. Barber and G. D. B. Kraan, *Biochim. Biophys. Acta*, 197 (1970) 49.
46. G. Ames and G. P. B. Kraan, reported at the "International Conference on the Photosynthetic Unit," Gatlinburg, Tennessee, 1970.
47. Cited in reference 48.
48. D. E. Fleischman, reported on "International Conference on the Photosynthetic Unit," Gatlinburg, Tennessee, 1970.
49. G. P. B. Kraan, G. Ames, B. R. Velthuys, and R. G. Steemers, *Biochim. Biophys. Acta*, 223 (1970) 129.
50. W. S. Lynn, *G. Biol. Chem.*, 243 (1968) 1060.
51. N. Nelson, F. Drechsler, and G. Neumann, *J. Biol. Chem.*, 245 (1970) 143.
52. G. Neumann, N. Nelson, and F. Drechsler, *Abstr. Int. Biol. Cong.* 11th, Seattle, 1969, p. 151.
53. B. Chance, *Fed. Proc.*, 26 (1967) 1341.
54. R. S. Crockwell, E. G. Harris, and B. C. Pressmonis, *Biochem.* 5 (1966) 2326.
55. E. C. Slater, *Eur. J. Biochem.*, 1 (1967) 317.

56. R. E. Mc Carty, *Biochem. Biophys. Res. Commun.*, 32 (1968) 37.
57. G. Neumann and A. T. Jagendorf, *Arch. Biochem. Biophys.*, 107  
(1964) 109.
58. E. A. Libermann and V. P. Skulachev, *Biochim. Biophys. Acta*,  
216 (1970) 30.

Figure Legends

Fig. 1. Spectrum of electron currents which are injected by excited bacteriochlorophyll into a single crystal ZnO-semiconductor electrode. Electrode potential: +0.5 Volt (measured against saturated calomel electrode); less than a monolayer bacteriochlorophyll deposited from benzene; electrolyte: 1N KCl +  $2 \cdot 10^{-2}$  M hydroquinone.

Fig. 2. Current-voltage dependency of photocurrents injected by bacteriochlorophyll.

Fig. 3. Simplified energy scheme of photosynthesis in green plants and bacteria which should visualize distribution of membrane-bound and soluble electron carriers: Carriers in squares indicate membrane-bound carriers, in dotted squares indicate carriers which are easily extractable from membrane or have little known behavior. Dotted areas indicate solution (electrolyte).

Fig. 4. Free energy-potential scheme of an array of electron transfer molecules (A, B, C, ....) and chlorophyll (Chl).

- a) when they do not form a complex (weak interaction between molecules);
- b) when they are forming a tight complex (strong interaction between molecules).

Fig. 5. Molecular redox elements as basic energy converting units in energy transducing membranes. (A, B) = complex of electron transfer carriers of different redox potential; Chl = chlorophyll;  $R_1$ ,  $R_2$  = reducing molecules; O = oxidizing molecule. Dotted area: solution (electrolyte); square: photosynthetic reaction center.

- a) scheme of redox element;
- b) free energy scheme.

Figure Legends (continued)

Fig. 6. Scheme to show how redox elements drive ion transfer and energy conversion.

a) structural arrangement of redox elements within the energy transducing membrane:

1) electron transfer complex; 2) chlorophyll (donor of low redox potential); 3) channel for ion transfer; 4) channel for energy conversion (driving of endothermic electrochemical reactions).

b) corresponding electrochemical circuit diagram:  $i_e$  = electron transfer current;  $R_e$  = internal resistance of electron transfer complex;  $i_i'$ ;  $i_i''$  = ion transfer currents;  $R_i$  = resistance for ion transfer;  $i_c$  = current of ions during parametric energy conversion;  $R_c$  = work resistance, at which electrochemical energy is convertible in a steady state way;  $C$  = membrane capacity; the stored electrochemical energy can be converted if the capacity is changed periodically (by conformation changes of the membrane).

Fig. 7. Influence of membrane potential on redox potential of membrane-bound electron transfer carriers. Selected example: Electron acceptor system of photosystem I in plant photosynthesis. Chl = chlorophyll; X = unidentified primary acceptor;  $Fd_M$  = membrane-bound ferredoxin; dotted area = electrolyte.

a) situation when suitable acceptor (soluble ferredoxin) is present for reduction.

b) when only an acceptor of much lower redox potential is present (MV = methylviologen). Methylviologen can be reduced after a sufficiently high potential ( $\psi$ ) has built up as a result of electron transfer to  $Fd_M$ .

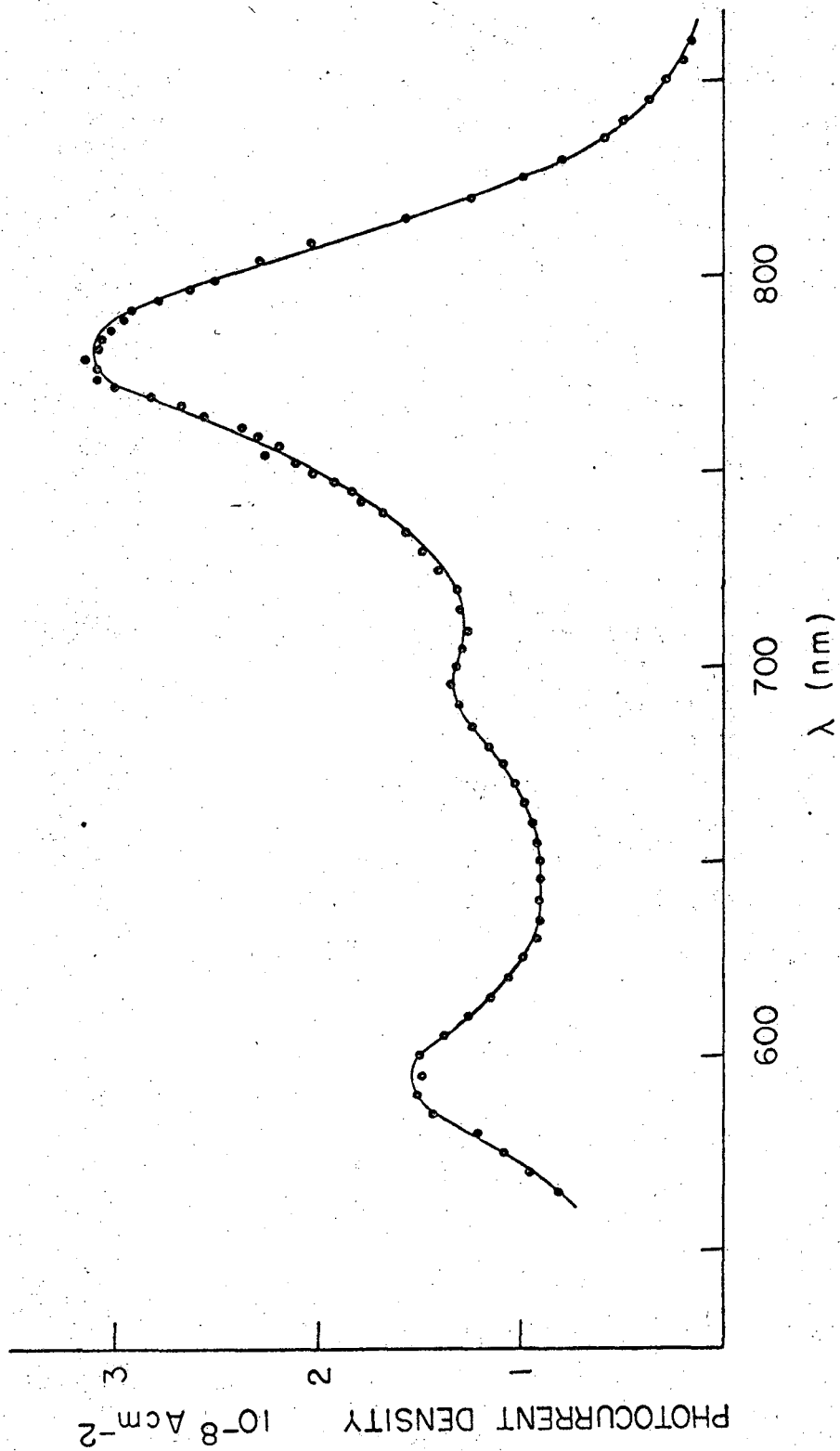


Figure Legends (continued)

Fig. 8. Energy conversion and energy flow in parametric energy coupling. Dotted arrows: reverse reaction.

Fig. 9. Capacitive energy-potential-diagram of a membrane which converts energy by parametric energy coupling: a) ideal case; b) real case, where nonlinear behavior of membrane and utilization of capacitive energy for mechanical work is considered.  $1 \rightarrow 2$ , electrochemical charging of membrane;  $2 \rightarrow 3$ , decrease of capacity (conformation change);  $3 \rightarrow 4$ , release of surplus capacitive energy as chemical energy;  $4 \rightarrow 1$ , restoration of original capacity (= conformation).  $1 \rightarrow 2' \rightarrow 3' \rightarrow 2$  ( $4'$ )  $\rightarrow 1$ : reverse conversion of chemical energy (ATP) into mechanical and electrochemical energy.

Fig. 10. Influence of membrane potential on delayed light emission from point x within the electron acceptor complex: 1) without potential; 2) with a potential which facilitates reverse reaction.



XBL 714-5147

Fig. 1

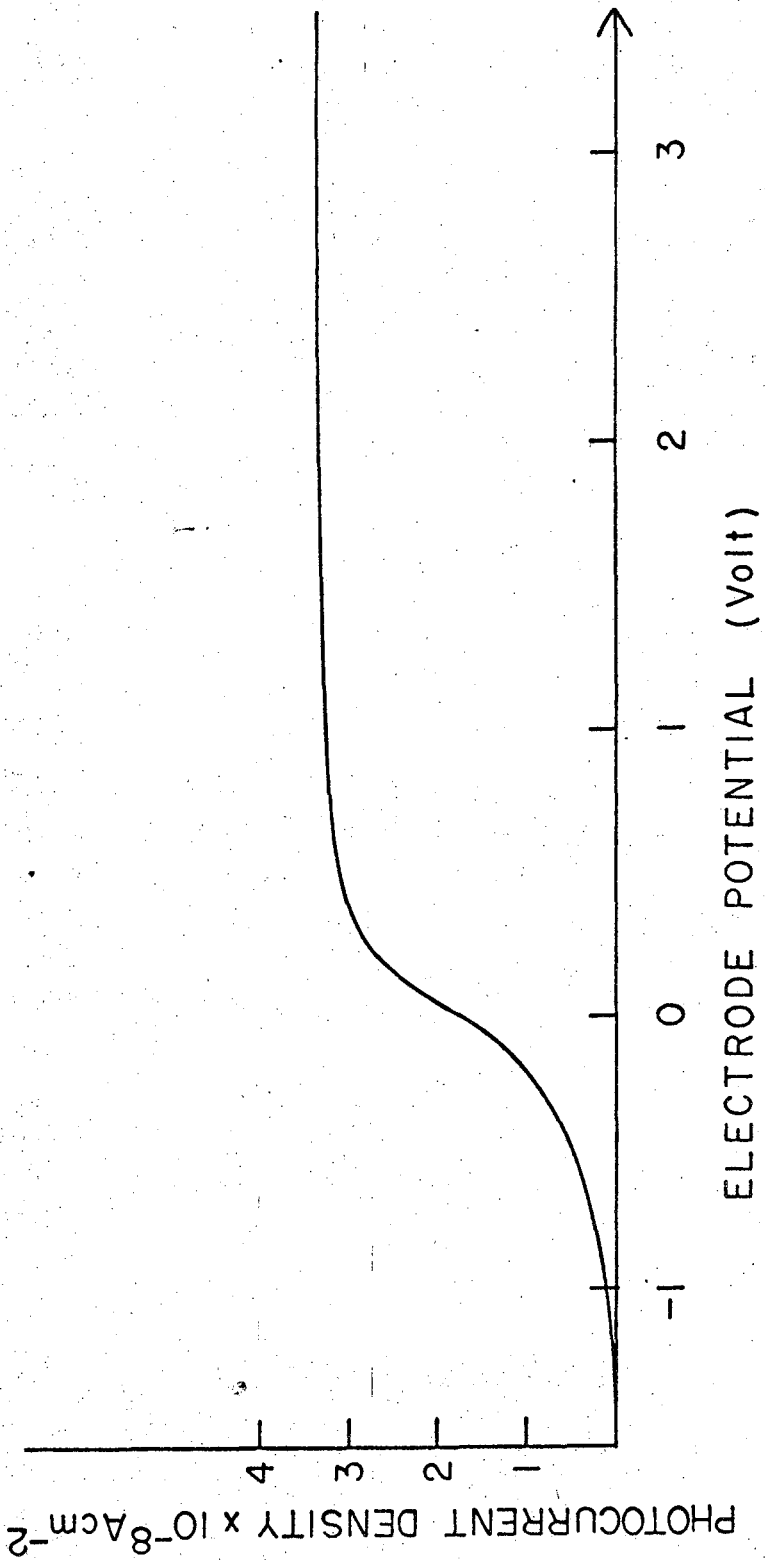
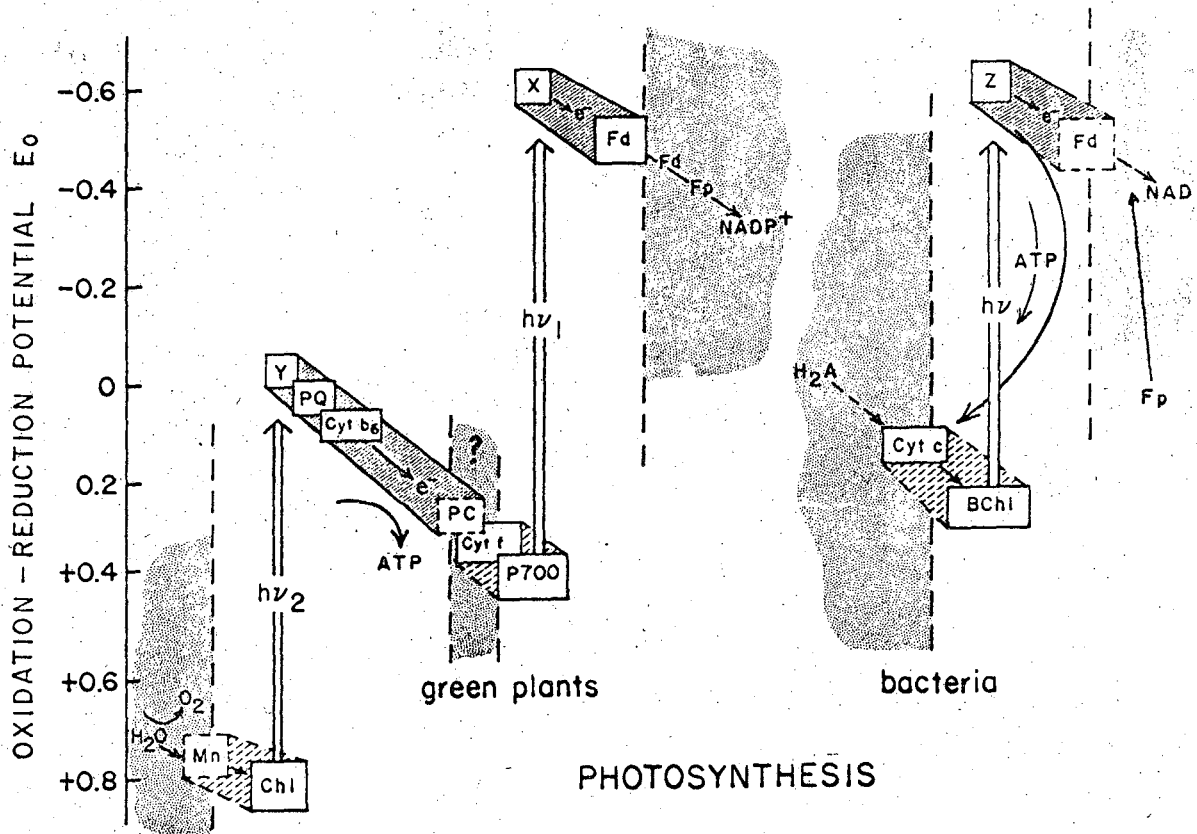


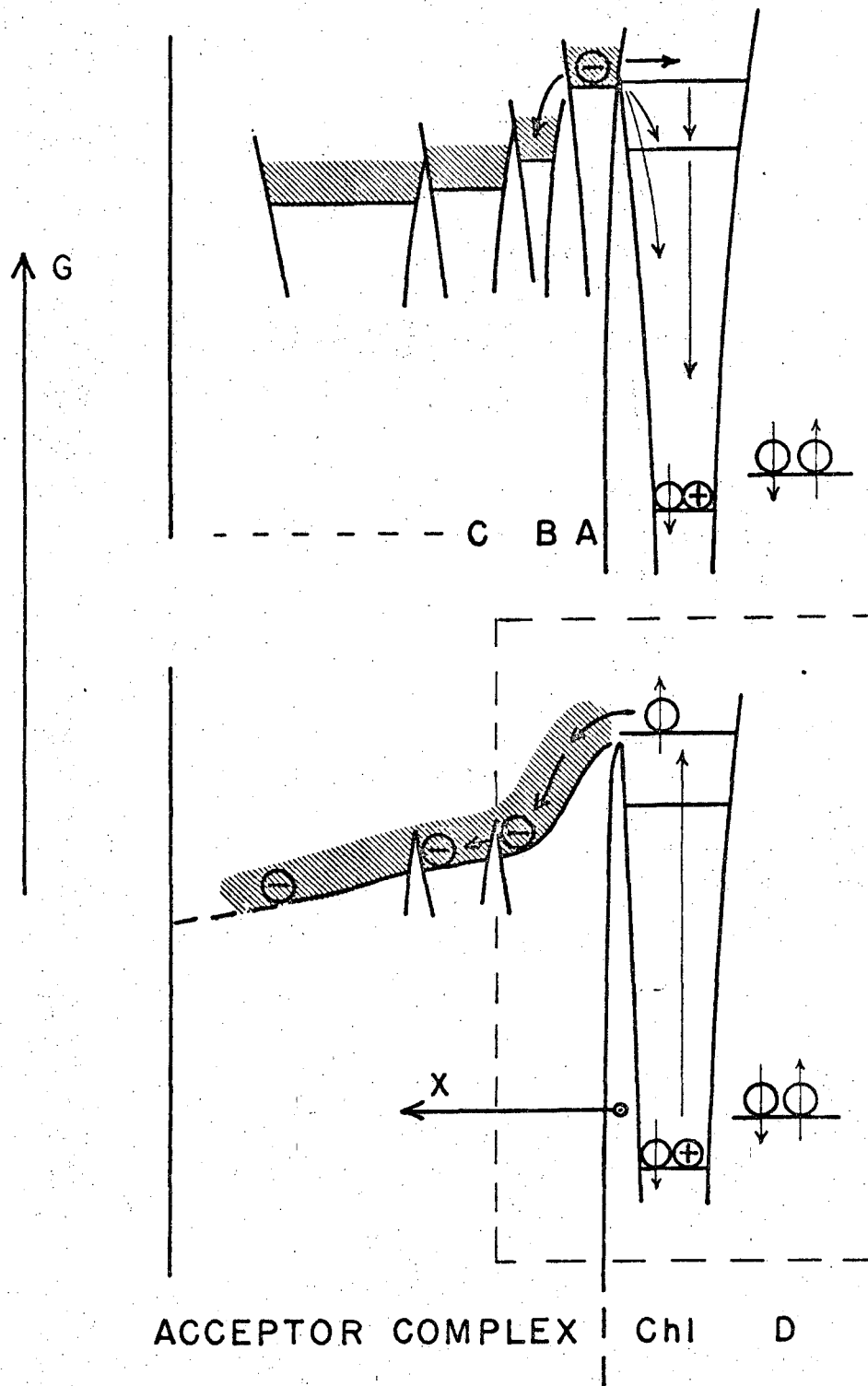
Fig. 2

XBL 714-5140



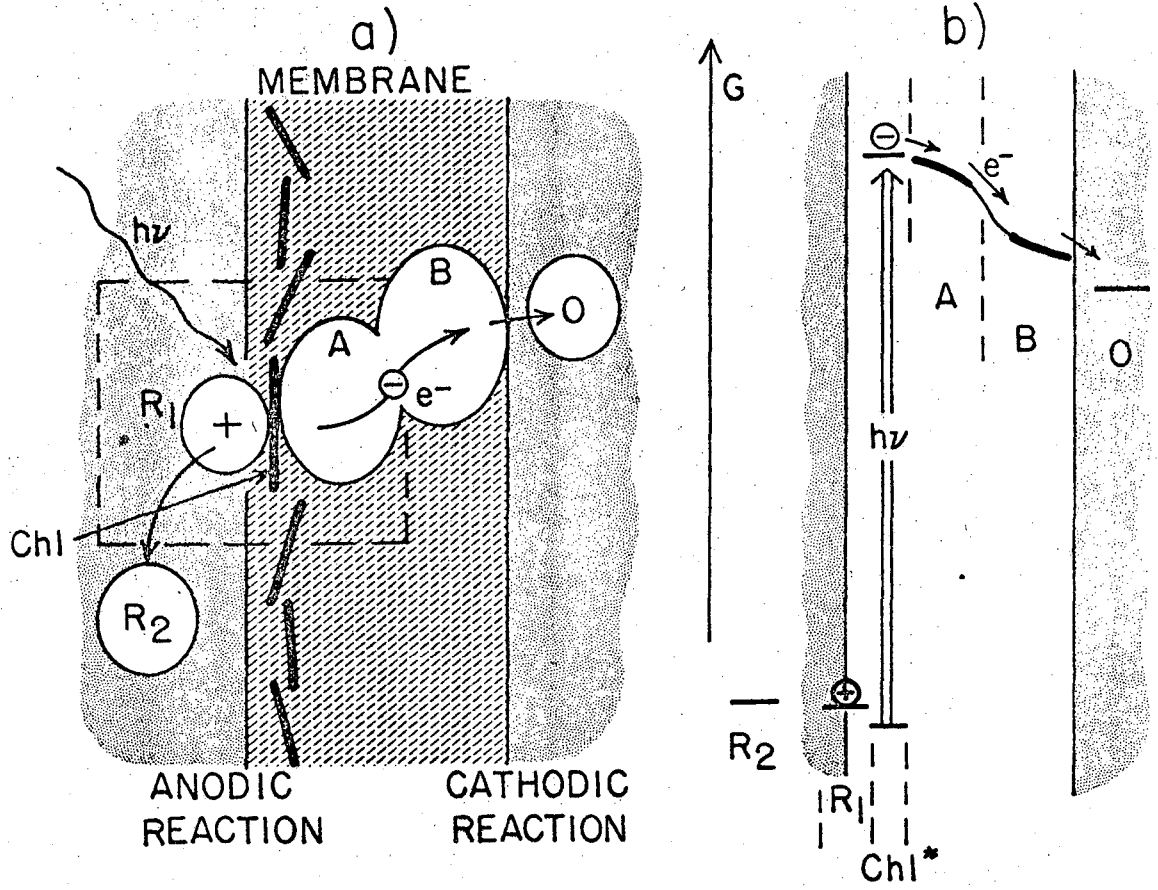
XBL 714-5144

Fig. 3



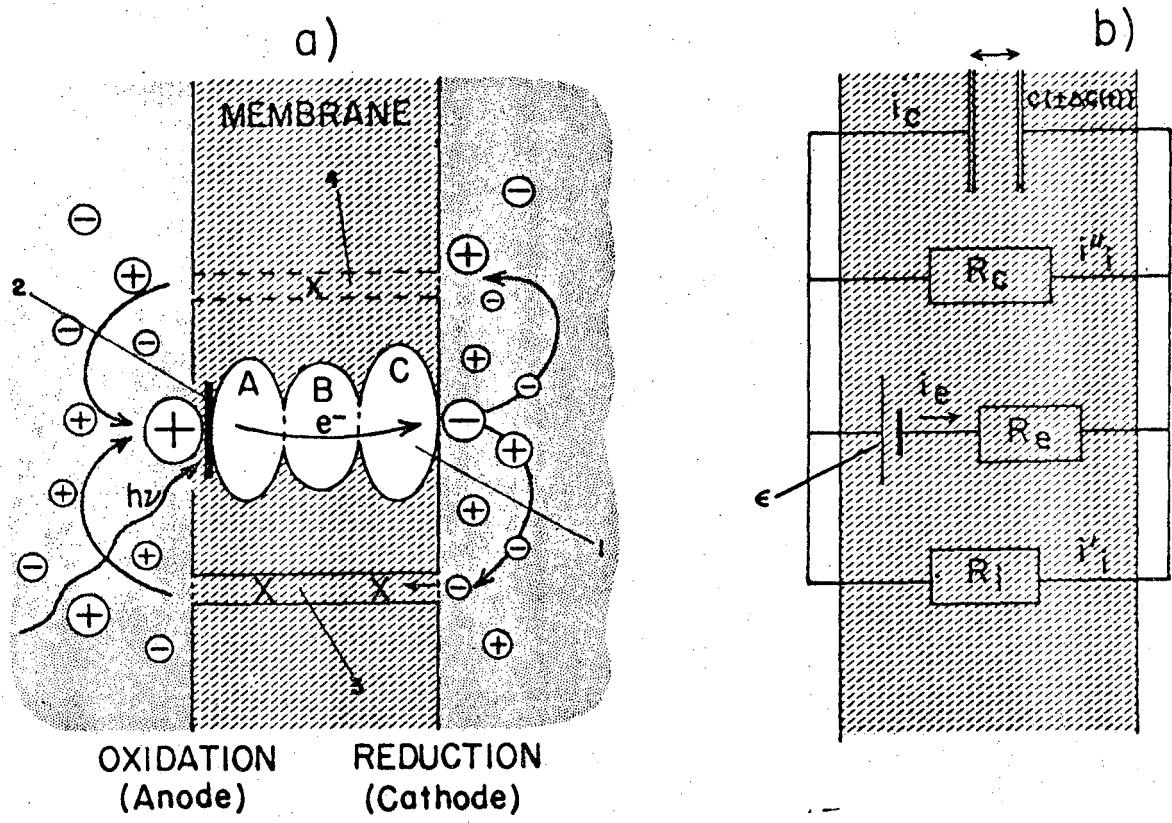
XBL706-5244

Fig. 4



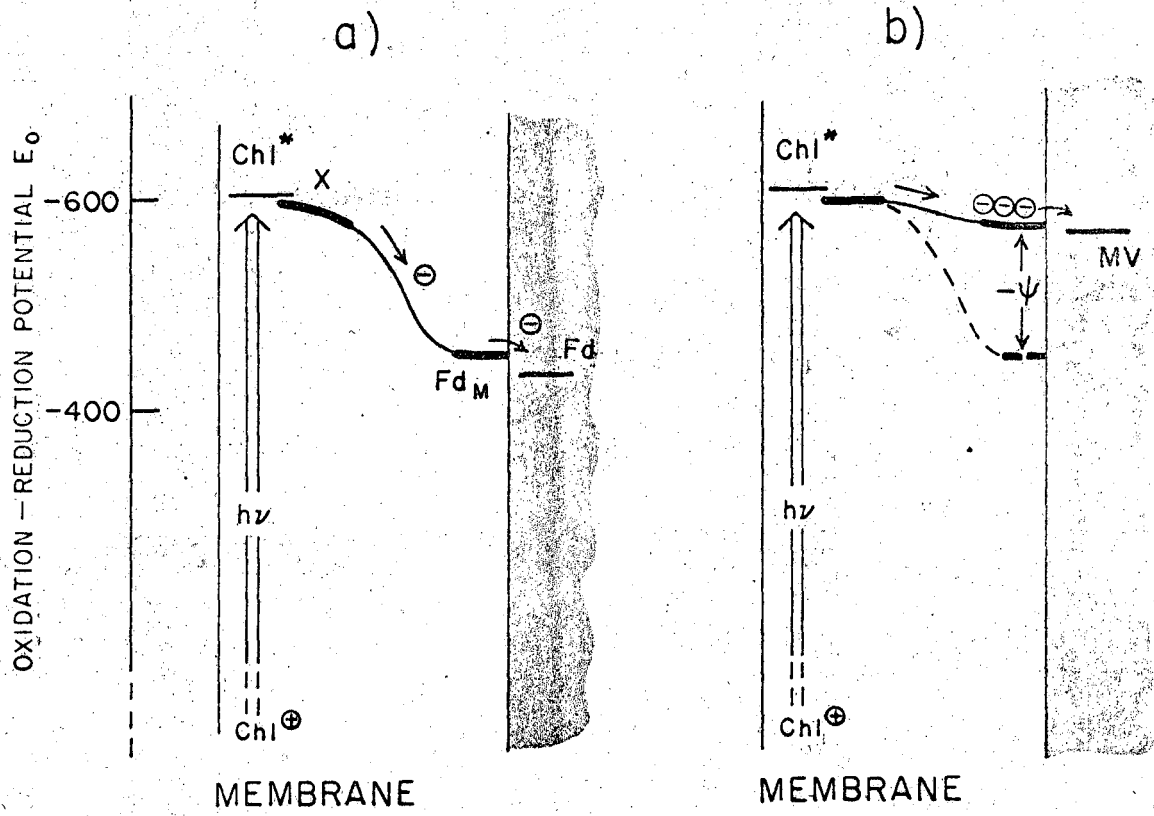
XBL 714-5141

Fig. 5



XBL 714-5142

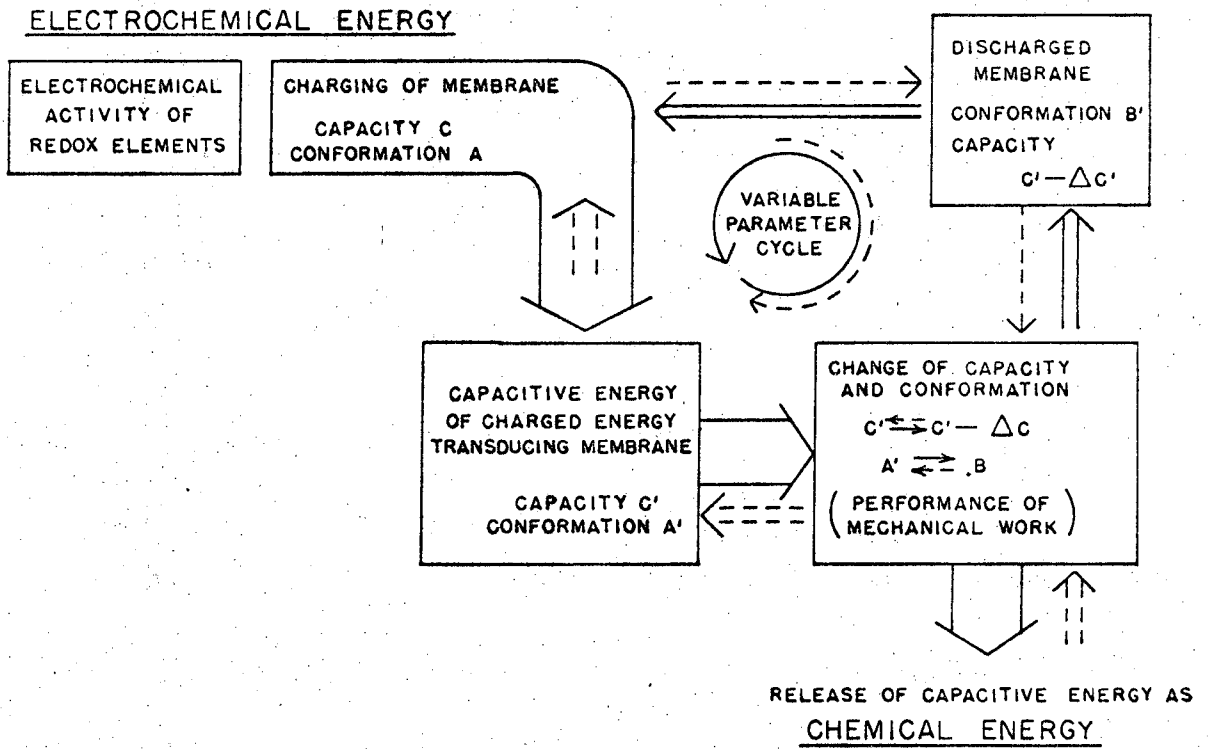
Fig. 6



XBL 714-5143

Fig. 7





XBL714-5146

Fig. 8

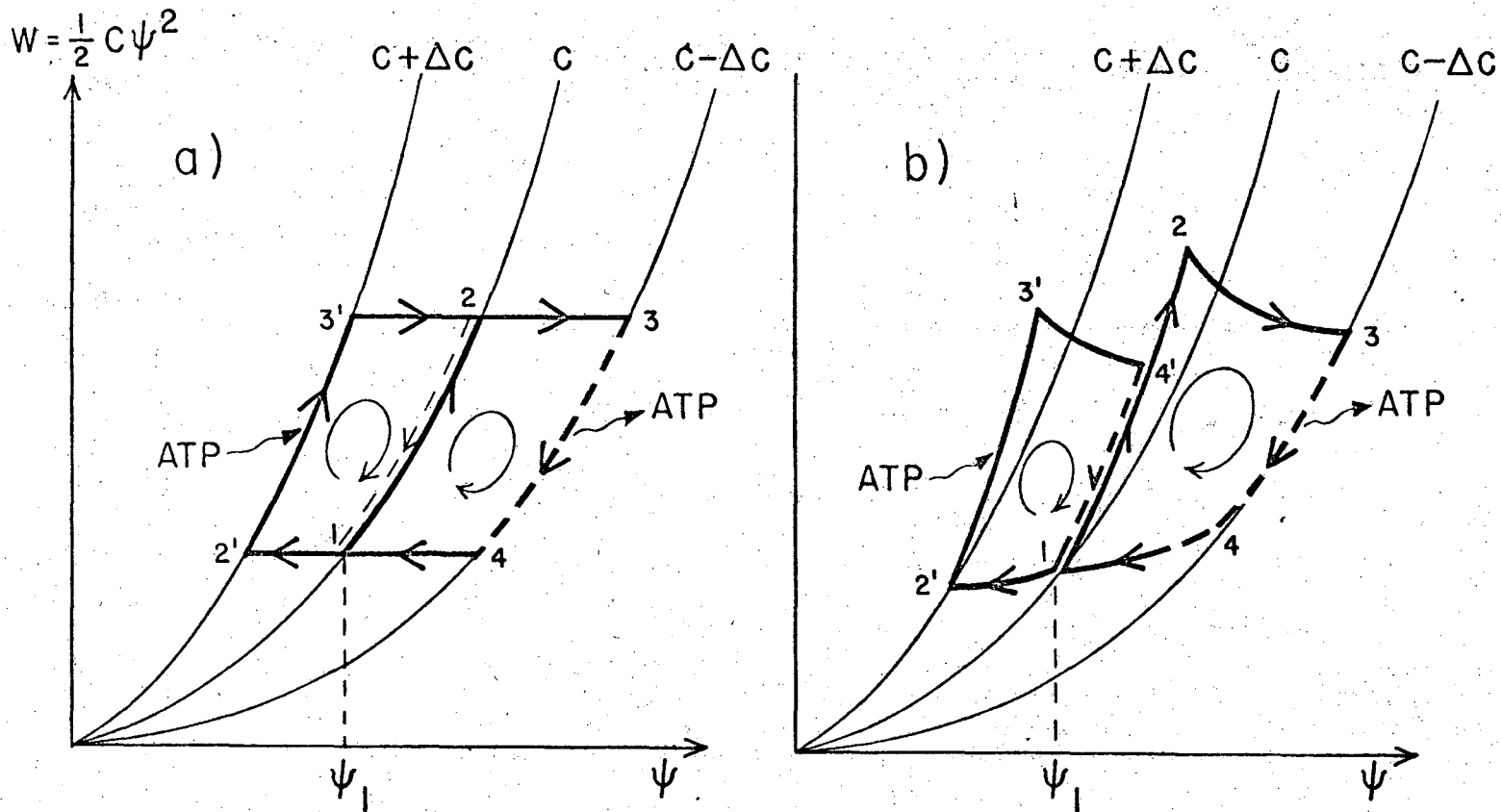
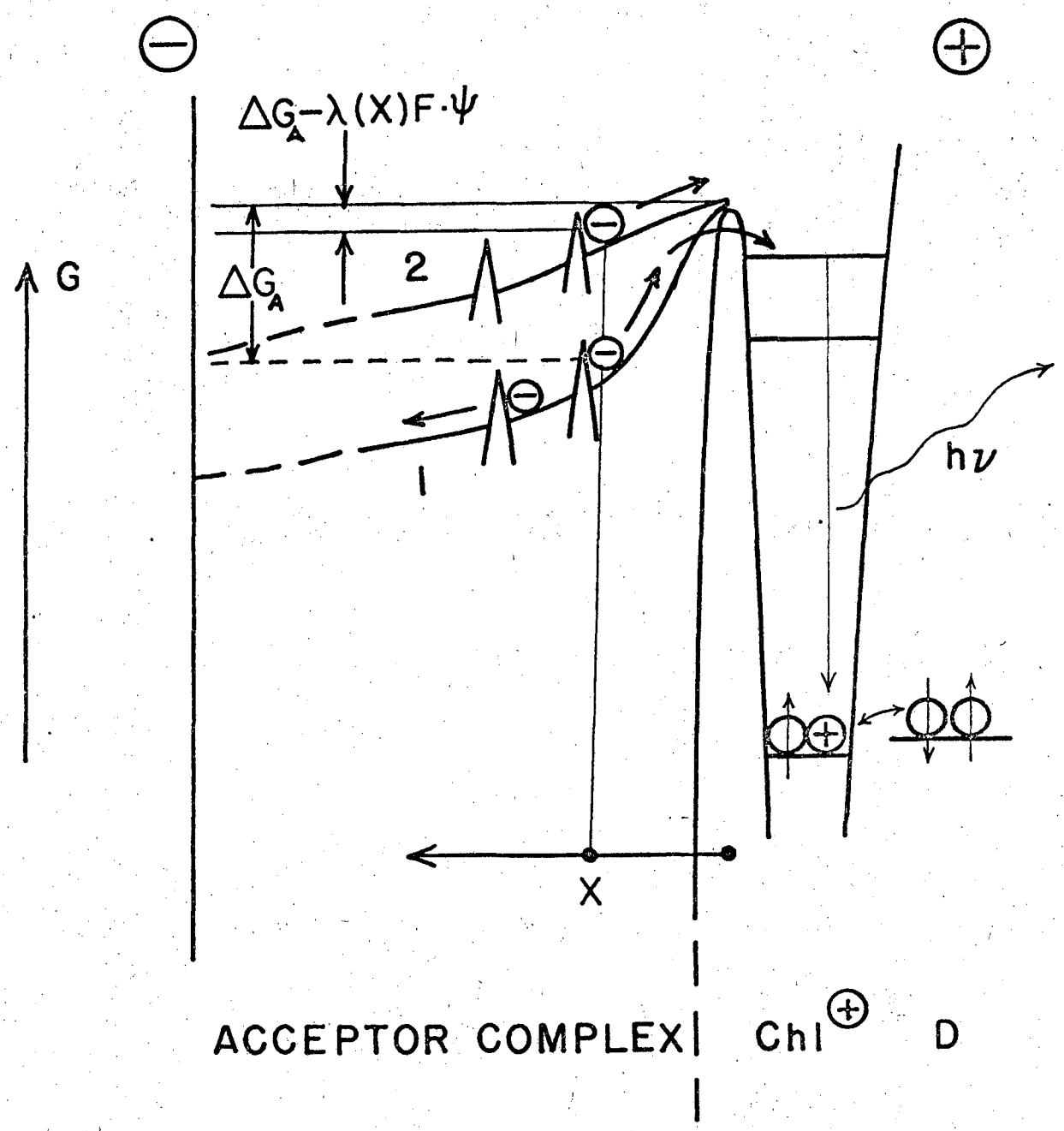


Fig. 9

XBL714-5145



XBL706-5241

Fig. 10

LEGAL NOTICE

*This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.*

TECHNICAL INFORMATION DIVISION  
LAWRENCE RADIATION LABORATORY  
UNIVERSITY OF CALIFORNIA  
BERKELEY, CALIFORNIA 94720