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A Proposal on the Function of Unsaturated Fatty Acids and Ionic Lipids: The Role of Potential Compaction in Biological Membranes

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Unsaturated fatty acids are constituents of nearly all biological membranes. They are always present in membranes which possess transmembrane potentials. Two completely different biosynthetic routes have evolved (aerobic and anaerobic) for placing *cis* double bonds in the 9 position on the fatty acids of membrane lipids. Bacterial membranes contain primarily *mono*unsaturated fatty acids, whereas eukaryote membranes contain a significant fraction of *poly*unsaturated fatty acids. The polyunsaturated fatty acids are concentrated in organelles, such as chloroplasts and mitochondria that are known to manipulate transmembrane potentials. I propose that the function of the unsaturated fatty acids is to facilitate the transmission of a local compaction of the membrane (in response to a transmembrane potential) laterally through the membrane. The role of the *cis* double bond at position 9 is twofold: first to create a kink in the chains of a large fraction of membrane fatty acids enhancing the separation of two regions in the membrane and second to enhance the rigidity of the membrane in the region between the head group and the 9 double bond. This ordered region contains those carbons proximal to the 9 carbon and which are in a regular array of *trans* conformations. The presence of a reasonable proportion of *cis* double bonds at position 9 will tend to maintain these *trans* conformations utilizing pi–pi (van der Waals) interactions between adjacent hydrocarbon chains at position 9. The disordered region contains the carbons distal to the 9 carbon. These have greater degrees of freedom and considerable *gauche* conformations. The role of the double bonds in the polyunsaturated fatty acids distal to carbon 9 is to facilitate trans bilayer pi–pi (van der Waals) interactions enhancing compaction of the bilayer during the electrostriction. I further propose that it is the function of the ionic headgroups to form an interlocking polyionic network which constitutes an elastic sheet. These ionic interactions would serve as the restoring force converting the compaction into a wave. The facilitation of the compaction of the bilayer together with the polyionic restoring force permits the membrane to transmit conformational changes from one transmembrane protein to another. Since transmembrane potentials are created and responded to by proteins each in a single location, it is thus proposed that a

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potential compaction wave emanates from the first protein in all directions in the plane of the membrane. The proposed wave would have both physical and electrical components. The electrohydrodynamic wave would require that the compaction oscillations be coupled to an oscillating electrical field. These proposals are applied to mitochondrial oxidative phosphorylation, and to transport across biological membranes.

1. Introduction

(A) UNSATURATED FATTY ACIDS IN BILAYERS

One of the striking disclosures of modern biochemical research is the relatively small variety of substances that dominate the composition of living cells and are ubiquitous among them. Most of these, including the amino acids, the nucleic acid bases, the sugars and the fatty acids are well understood. They are understood not only in the context of their assembly into arrays, which may be polymeric (nucleic acids and proteins) or molecular associations (such as the bilayer) but also as to the function of those arrays. The modern biochemist can identify the roles of each portion of every one of these small molecules and many more complex ones, such as the vitamins. The role of the *cis* double bond in the unsaturated fatty acids has intrigued lipid chemists for many years. It lowers the transition temperature of the membrane thereby enhancing the fluidity and disorder within the membrane. This effect is not, however, restricted to position 9 and thus does not explain the uniqueness of the 9 *cis* double bond.

The *cis* double bond in the 9 position is so significant to living cells that primitive life forms used anaerobic processes for its biosynthesis. With the advent of atmospheric oxygen and aerobic metabolism, a completely different and novel biosynthetic pathway evolved (Bloch, 1969). All living cells contain fatty acids with this double bond although in some bacteria these may be replaced by fatty acids with a cyclopropane group in the same position.

Most *poly*unsaturated fatty acids found in membranes have *cis* double bonds distal to that in position 9. The occurrence of membrane lipids in a bilayer first lucidly proposed by Gorter & Grendel (1925) and by Danielli & Davson (1935) was demonstrated by Branton (1966).

The first observation regarding the precise arrangement of hydrocarbon chains in a biological membrane was obtained by Engelman (1970). He found using X-ray diffraction on membranes obtained from *Mycoplasma* that the hydrocarbon chains were arranged in a hexagonal array whether the membrane was below or above its transition temperature. The observable

difference was in the spacing which shifted from 0·42 nm to 0·46 nm. The hexagonal array for hydrocarbon chains was first described by Muller (1930). His studies were on crystalline paraffins. The hexagonal array refers to the packing array of hydrocarbon chains when viewed in a plane perpendicular to the axis of the chains. Thus each chain is surrounded by six chains which form a hexagon around it. The hexagon is not a symmetrical one in the crystalline state. This is due to the fact that the hydrocarbon chains are in an all-*trans* conformation and may therefore be viewed as an ellipse from the end of the chain. Close packing of such ellipses forbids a symmetrical hexagon surrounding each chain. He later showed (Muller, 1932) that for long chain hydrocarbons (C_{16} and greater) there was a transition temperature increase of spacing from 0·42 nm to 0·46 nm. A different type of transition temperature exists for short chain hydrocarbons. Mueller (1930) also found that the transition to 0·46 nm spacing was accompanied by the formation of a *symmetrical* hexagonal array. He proposed that this was possible because of oscillations or rotations of the molecules around their chain axes. This is one mode of thermal agitation. It is important to note that these transition arrays only applied to very long hydrocarbon chains and that Garner, van Bibber & King (1931) noted that in a molecule containing 34 carbon atoms the nature of the headgroup determined the mode of packing!

These observations make the recent deuterium and [^{13}C] NMR data of Seelig & Seelig (1974) most intriguing. They found that in phospholipid bilayers containing saturated hydrocarbon chains the first nine to ten carbons are in the *trans* conformation. Beyond that the frequency of *gauche* conformations increase sharply enhancing the disorder in the central region of the bilayer. It should be noted that this observation applies to saturated chains with zwitterionic headgroups. The headgroups appear, therefore, to enhance the *trans* conformation frequency in the range of the first nine to ten carbons but are considerably less effective beyond that point in the chain.

Smith *et al.* (1977) have extended these experiments to include the same membranes used by Engelman (1970) in his X-ray studies. The *Acholeplasma* membrane contains unsaturated fatty acids and also cholesterol. The latter (and the plant sterols have similar structures) is rigid in the region of its rings and flexible on its side chain. Its orientation in the bilayer with the hydroxyl hydrogen bonded on to some portion of the polar head groups or to water would contribute rigidity to that region between carbons 2 and 9 of the neighboring acyl chains and yet permit flexibility in the interior disordered region. It is perhaps not coincidental that the rigid part of the molecule terminates at about carbon 9 of the fatty acid chains when placed in a model of the bilayer (Vandenheuvel, 1965; see also Rothman & Engelman, 1972). It

is interesting that the methyl group at position 21 of cholesterol pushes the side chain (attached to position 17 on the *D* ring) out of line with respect to the series of rings producing a kink in this same region of the membrane.

The presence of the 9 *cis* double bond in a large fraction of the chains would be expected to have two important effects on the conformations of the acyl chains in the membrane. The first is that it would increase the frequency of *gauche* conformations (of even the neighboring saturated fatty acids) at and beyond the 9 carbon of these acyl chains. The *cis* double bond itself is at 120° with the axis of the chain above and so it would (in all those chains that contain it—including the polyunsaturated fatty acids) introduce a kink at the same point in the membrane. It would therefore enhance sharply the disorder beyond carbon 9 toward the center of the bilayer.

The second proposed function for the *cis* double bond at position 9 is that of interacting with other double bonds at the same position by pi–pi (van der Waals) interactions. The effect of such interactions would be to enhance the rigidity of the first nine *trans* conformations on the same acyl chains. Thus the headgroups may act to rigidify the proximal region of the chain as observed by Seelig and Seelig and the *cis* double bond may enhance the rigidity in this region and yet sharply terminate it introducing disorder beyond the 9 carbon of the chain.

Where *poly*unsaturated fatty acids occur in a membrane, the *cis* double bonds distal to that on carbon 9 are in the disordered region of the bilayer interior. Each double bond is a plane with free rotation (with respect to the adjacent plane) around the methylene between the double bonds. The second double bond and all subsequent double bonds have the capacity to be parallel to the plane of the bilayer. In this region of the bilayer it is thus possible for double bonds of fatty acids on one side of the bilayer to interact with those on the other side. The van der Waals (pi–pi) interactions of such double bonds are about 5–10 times as strong as those between methylenes.

(B) IONIC HEADGROUPS IN BILAYERS

All biological membranes that manipulate potentials contain lipids with ionic headgroups. Many of these headgroups in phospholipid membranes are zwitterionic containing both positive and negative ions. Some headgroups lack positive charges and are therefore acidic lipids. It is suggested by the work of Hargreaves & Deamer (1978) and from Gebicki & Hicks (1976) that the acid membranes are stable because there are spacers separating the charged headgroups. In some of these membranes half of the anions are protonated at the surface of the membrane, which has unusual acidity at its surface, and these act as spacers between their anionic neighbors. In either system the headgroups are required to remain in an

interlocking ionic system. The properties of the acidic lipid in this system, must surely be different from that of the zwitterionic lipids. This is emphasized by the requirement for a constant ratio of zwitterionic to anionic lipids in a variety of membrane systems (Pluschke, Hirota & Overath, 1978).

Engelman's (1970) X-ray diffraction data on *Acholeplasma* show that the hexagonal array describes the hydrocarbon packing in this membrane has one other important implication. His observation must be coupled with the fact that the phospholipids (anionic in *Acholeplasma*) have two chains. For the zwitterionic lipids, one negative (tetrahedral) phosphate and one positive (tetrahedral) ammonium. Since these charged groups are approximately the same size as the cross-section of the chains themselves, one interpretation of these data is that the charges form an interlocking pattern on the surface of the bilayer. Arrays are easily assembled from models because the two charges on each molecule form a six-membered stable ring. Two other features of the nature of the charges on the lecithin molecule inhibit charge repulsion between otherwise adjacent charges. The quaternary nitrogen has the positive charge and it is shielded by neutral methyl groups. The phosphate, on the other hand, has its negative charge distributed between two oxygens each of which are at the corners of the phosphate tetrahedron. This feature of the distributed negative charge enhances the coupling of two cations to the single anionic phosphate.

Such an interlocking system has several obvious implications. The strength of the ionic interactions would contribute maximal stability and rigidity to the hexagonal array of the hydrocarbon chains beneath them in the bilayer. The headgroup ionic interactions thus maintain a rigid *trans* conformation for the first 9 carbons of the chains. This is consistent with both the NMR and the spin label data as discussed by Seelig & Seelig (1974) and also by Smith *et al.* (1977).

The ESR data discussed in both papers indicates that the closer the methylene is to the headgroup the less disordered (the fewer the *gauche* transformations) is the hydrocarbon chain at that carbon. The observation that the spin label perturbs the system (in contrast to the NMR) as it approaches the headgroup strengthens this view. It implies that the forces at the headgroup which favor the *trans* conformations are stronger than the steric repulsion of the inserted nitroxide group.

A second important implication of the interlocking ionic headgroup array is that it maintains the chains in a fixed height perpendicular to the plane of the membrane. This implies that the double bonds at position 9 are always held at the same position with respect to the ionic sheet. Seelig has also found that the two acyl esters (primary and secondary) are arranged differently with respect to the plane of the membrane. If the 9 double bond were to be

arranged by the headgroups in a sheet within the membrane then it should be on only one of the two chains otherwise they would be at different heights perpendicular to the membrane. Most phospholipids have saturated fatty acids at position 1 and unsaturated fatty acids at position 2 of the glycerol. That the 9 double bond is at the same height normal to the membrane permits it to interact with neighboring 9 double bonds in pi–pi interactions. This in turn renders order to the chains above it and sharpens the division between ordered and disordered regions.

A third important feature of the interlocking ionic array is that it is minimally interlocked. This maximizes the lateral fluidity (Lee, Birdsall & Metcalfe, 1974) while maintaining the interlocking ionic charges. If both the cation and the anion were each localized at a single atom, the ion pair at each headgroup would not interact with its neighbors. There could be no interlocking quality among adjacent lecithin molecules. If both were bidentate the lipid molecules would have considerably greater restrictions on their lateral motion and less lateral mobility. The fact that the anion has its charge divided between two oxygens which will interact with two different headgroup nitrogens (each with half the attractive force of a molecule). Its capacity to exchange partners is enhanced by the weaker interactions.

A fourth feature of the proposed interlocking ionic system is its elasticity. The system, if stretched out of plane, will snap back because stretching implies increasing the distance between the charges.

Seelig & Seelig (1974) have emphasized (and this author agrees) the distinction between disorder and fluidity. Disorder is concerned with the lack of regular array in a system and fluidity refers to the rate of motion. There is no basis for assuming that any rates are associated with the presence or absence of arrays. However, the nature of the motion in an ordered system will be different than that in a disordered system. The ordered region of the membrane would appear to permit molecular motion perpendicular to the plane of the membrane with an elastic restoring force. The restrictions of the all-*trans* conformation between the headgroup and the 9 double bond makes the molecule "stick-like". It may "bob" and it may move laterally in the hexagonal array (with its headgroup) but it will not contribute to a thinning of the membrane by folding. Indeed, it will rigorously render a paraffin quality to the membrane in this region.

In contrast the disordered region beyond the 9 double bond *allows* a thinning of the membrane by the introduction of *gauche* conformations as illustrated by models in the discussion of Seelig & Seelig (1974). Such thinning is accentuated by double bonds in the *poly*unsaturated fatty acids and would be even further enhanced by pi–pi interactions. Double bonds distal to that on carbon 9 may be parallel to the plane of the membrane.

The membrane described above has a paraffin barrier to the aqueous environment that it faces on both sides. This barrier is restricted to a remarkably short transmembrane region. It includes only eight or so carbons on the hydrocarbon chains. The resistance that this region has to bending and thereby to thinning of the membrane would be even more important if the membrane were rendered thinner by external forces. Thus this region might retain its paraffin barrier against vigorous motions of a fluid interior. This quality is due to the restrictions imposed upon the nature of the motions available to the chains in this region of the bilayer.

(c) MEASUREMENTS ON MEMBRANE THICKNESS

One might anticipate that impressing a potential across a membrane would cause an electrostriction on the bilayer. This is because the positive and negative charges are separated by only 50 or so Angstroms and the field is therefore very large. Many attempts have been made to establish electrostriction by impressing a voltage on a bilayer membrane. These electrostriction measurements have been made by impressing the charge on the entire membrane.

The thickness of a bilayer membrane was actually first determined by Fricke (1925) in the same year that Gorter & Grendel (1925) published their classic paper. Fricke measured impedance and concluded from Langmuir's (1917) data that the red cell membrane was 30 carbon atoms thick! He assumed the dielectric constant was 3.

Most measurements of membrane thickness have been conducted on the planar bilayer system (BLM) developed by Mueller *et al.* (1962). Upon impressing a potential across the bilayer the specific capacitance (White, 1970) and the total capacitance (Babakov *et al.*, 1966; Lauger *et al.*, 1967; Rosen & Sutton, 1968) both increase. This increase in capacitance is indeed attributed to a thinning of the membrane (White, 1970; Fettiplace *et al.*, 1971; Lauger *et al.*, 1967). This interpretation of thinning has come under criticism (Wobschall, 1972) because microlenses of solvent alkanes that form in the bilayer under these conditions might affect both the capacitance and the area. The studies conducted by Babakov *et al.* (1966) were done on the Mueller type BLM. There was presumably solvent present. They do not indicate the type of lipid they used to make the bilayers. There was an increase of capacitance upon the impression of 0·1 V potential across the membrane. They interpreted the increase in capacitance as due to an increase in area since they used the refractive index of monochromatic light and Brewster's angle to estimate the thickness of the bilayer. They found $7{\cdot}4 \pm 1{\cdot}5$ nm as the thickness and noted no change upon impression of a

potential. This large value might also be due to microlenses of alkanes. Huang & Thompson (1965) had originally measured the thickness of the BLM ($7{\cdot}2 \pm 1{\cdot}0$ nm) using this technique. Lauger *et al.* (1967) used egg lecithin in their films and ascribed the increase in capacitance to a thinning of the membrane. He obtained a maximal increase of capacitance "of about 10%" as the voltage across the membrane was increased. The experiments of Rosen and Sutton verified the increase (up to 15%) with egg lecithin films, but they did not ascribe it to a thinning of the film. They left it unexplained.

White (1970) observed the capacitance increase with "oxidized cholesterol" and later with lecithin containing acyl chains of cyclopropane (bis-dihydrosterculoyl) fatty acids (White & Thompson, 1973). In the latter paper, White and Thompson discuss six uncertainties with regard to the measurement of changes of membrane thickness by changes in capacitance of the membrane in the presence of an impressed potential. It appears that their increase in capacitance with increased potential is a result of both a thinning of the membrane and an increase in area.

Alvarez & Latorre (1978) have found a very small (less than 0·2 nm) thinning of the membrane in similar bilayers containing unsaturated fatty acids.

The above measurements have all been made by impressing the voltage on the entire membrane. The thinning of a membrane is accompanied by an increase in its area. No measurements have been made on the increase in area of a bilayer on which a potential has been impressed. This is probably best done with a vesicle which would enlarge. The implications for water flow and other aspects of biological membranes are obvious.

2. Theory

(A) THE MOST GENERAL FORM

A consideration of the biological significance of the thinning of a membrane in response to an applied potential is not based upon the *overall* thickness of a membrane in the presence of the field. A potential is not impressed upon a biological membrane by external electrodes. Indeed, a potential is impressed at a single point in the membrane by a protein which appears in all known systems to be a transmembrane protein (DePierre & Ernster, 1977). Such a "point compaction" (which we shall call a potential compaction) may be visualized as the dropping of a stone in a lake. The stone "compacts" the lake at a single point. Once compacted, the water propagates the wave away from the point of compaction. The energy required to create the local compaction is negligible when compared to that

required to compact the lake! The membrane, albeit a liquid, is only two molecules thick. Any compaction it underwent must be facilitated by conformational changes in its molecules. A compaction or thinning of the membrane necessarily requires that the distance between the molecules in the plane of the membrane be increased since they must occupy the same volume. Thus the spacing must increase and the membrane must spread locally.

It should further be noted that the impression of a potential at a single point in the membrane can occur whether or not there is a pre-existing overall transmembrane potential. Indeed, it may neutralize or reverse such a resident (or resting) potential locally and have the same effects.

A potential compaction created in a membrane that consists of lipids with zwitterionic headgroups and unsaturated fatty acids may result in a compaction wave that emanates from the point of compaction. If the fatty acids are monounsaturated then some of the energy that created the initial compaction is expended in overcoming the viscosity of the chains in the region beyond the 9 *cis* double bond network. If the fatty acids are polyunsaturated then the system has in addition the pi–pi (van der Waals) interactions across the bilayer to enhance the propagation of the wave. The difference between these two, according to this hypothesis, is related to the kinetics of the transport proteins involved. The polyunsaturated fatty acids may thus permit more rapid kinetics of the ion transporting proteins.

In this manner the transmembrane potential, the unsaturated fatty acids, and the ionic headgroup lattice have together permitted the first transmembrane protein (that created the transmembrane potential) to communicate a conformational change to a second transmembrane protein. Transmembrane proteins are subject to conformational changes in this circumstance because they are anchored into the two ionic sheets on either side of the bilayer. The conformational change that the second protein undergoes will be in that portion of the protein inside the hydrophobic region of the bilayer.

Let us suppose that the second protein transports an ion across the membrane as a consequence of its conformational changes (which were in response to the potential compaction) and that the migration of the ion across the membrane neutralizes the charge created by the first protein. Under these conditions the local transmembrane potential due to the first protein shall have both created a field which provided the energy to transport the ion and compacted the membrane which caused the conformational changes that facilitated the ion transport. The second (ion transporting) protein shall have both transported the ion and collapsed the field created by the first protein, thereby reversing the compaction (or

expanding) the membrane. Indeed, if the potential creating protein (the first) created the transmembrane potential most efficiently at the crest of the wave (membrane in the expanded state) and the responding protein transported the ion most efficiently at the trough of the wave (membrane in the compacted state) then the system would be in resonance. Such a membrane would have the potential creating process coupled most efficiently to ion transport.

Along with the physical property of expansion and contraction, the system has become an oscillating electrical field. Despite the relatively low voltages that are associated with membranes and transport (generally 50 to 250 mV) the fields are enormous because the voltage is applied over only 7·0 to 8·0 nm. A large oscillating field implies significant effects on the charged regions of the proteins. As an example, let us say that a protein or a fragment of a protein contains in one region (or arm) a high concentration of positive charges and in another region a high concentration of negative charges (the second region may merely be anchored into the membrane matrix). Such a protein in an oscillating electrical field could undergo substantial conformational changes. Furthermore, these changes would be in unison with the mechanical wave properties of the membrane. At least two transmembrane proteins, bacteriorhodopsin (Shinar *et al.*, 1977) and retinal rhodopsin (Cone, 1972), have been shown to have large dipole moments. The latter is of the order of 700 D. These measurements were made on detergent solubilized protein so that the actual dipole moment in the membrane remains unknown.

All membranes that have potentials coupled to ion transport (including protons), require unsaturated fatty acids. This includes the mitochondrion, the chloroplast, the sarcoplasmic reticulum, bacterial transport of ions, the brush border membrane of the mammalian intestine and numerous others. On the other hand, not all ions that are transported across lipid bilayers require unsaturated fatty acids as bilayer components. Racker & Hinkle (1974) have shown that bacteriorhodopsin containing vesicles of saturated fatty acid phospholipids pump protons in response to light below the transition point. The energy source for the ion transport is a photon. It is important to note that the same protein that absorbs the photon, pumps the proton. In this system, communication between two proteins is not necessary and therefore according to this hypothesis, unsaturated fatty acids are not required.

(B) THE WAVE

Let us examine the wave that results from this hypothesis. Waves have several features that together describe them. These include the frequency, the

wavelength, the velocity, the amplitude, the damping characteristics and the source of energy required for their propagation. The manner in which the proposed wave is generated and its function will differ in each biological membrane. Nonetheless, the hypothesis suggests a number of features that would apply in each case.

The source of energy for the wave must be related to the first protein that creates the potential compaction. It may be a photon or it may be substrate cleavage each associated with the movement of an ion (or an electron) across the membrane. The kinetics of this transport establishes the frequency of the wave. It is the turnover number of the transport protein.

A principal feature of this hypothesis is that one transmembrane protein can communicate with a second transmembrane protein in the same membrane without physical contact between the proteins by utilizing membrane potential, unsaturated fatty acids and ionic headgroups. The distance in the plane of the membrane between these proteins is half the wavelength of the wave provided the proteins are in resonance. This requirement in turn is based on the first protein transporting ions or electrons only when the membrane is in the expanded state and the second protein only transporting when the protein is in the contracted state.

In a moving wave the product of the frequency and wavelength is the velocity. In some membrane systems such as the nerve these three may be independently determined. In a standing wave in a lattice the frequency and wavelength are independent of each other. The standing wave has regions of maximal amplitude (where the molecular motion is at a maximum) and nodes. In this hypothesis such maxima must be at the two transmembrane proteins. The nodes would be half way between them. One would expect short saturated fatty acid chains to migrate to the nodes.

The amplitude of the wave has two interesting aspects. On the one hand it determines the extent of the conformational changes experienced by the proteins. On the other hand it describes the usefulness of double bonds beyond the 9 carbon in the polyunsaturated fatty acids in facilitating the wave. Thus one would anticipate that the magnitude of such a wave in mitochondria which contain largely linoleic acid would be greater than that in chloroplasts which contain largely linolenic acid. The latter have the most distal double bond closer to the methyl end of the chain and the transmembrane reach would therefore be shorter.

The damping characteristics of the wave are necessarily related to the microviscosity and molecular frictional properties of the system. One would anticipate, however, that the presence of polyunsaturated fatty acids in the membrane would inhibit damping of the wave. This is because the transmembrane pi–pi interactions would facilitate the compaction process

thereby opposing the restoring force which is provided by the ionic lattice and the 9 double bond pi–pi interacting lattice. The enhancement provided by the polyunsaturated fatty acids therefore lowers the dissipation of the energy which created the compaction. Finally, the polyunsaturated fatty acids would increase the frequency of the wave. Thus the potential creating protein may now have a higher turnover number.

The oscillating electrical field has a further important implication concerning the enzymes and transport proteins within the membrane. All of the dipoles on each protein in the membrane must oscillate with the field. This requirement is a most important conclusion with regard to the kinetics of both the ion transporting proteins and the enzymes anchored to the surface of the membrane. For an enzyme to engage in enzymatic activity independently of the oscillating field it would necessarily be removed from the immediate surface of the membrane.

(C) POTENTIAL COMPACTION AND OXIDATIVE PHOSPHORYLATION

It was Peter Mitchell (1961, 1966) who first proposed that electron transport in the inner mitochondrial membrane (and in the chloroplast) is connected to ATP synthesis by both membrane potential and a ΔpH (pH difference across the membrane). The statement was based upon a proposal of Lundegardh (1945) which in turn was a development of the observations of Lund (1928) and of Stiehler & Flexner (1938). Mitchell's hypothesis combined the observations of these workers with the development of the fuel cell in which hydrogen ions are produced on one side and consumed on the other. It requires that the membrane be impermeable to protons, that there be a transmembrane potential, that membrane components that are coupled—be asymmetrically organized on the membrane, that protons moving across the membrane have the capacity to drive ATP synthesis, and that the membrane itself be essential for coupling electron transport to ATP synthesis.

Since Mitchell has made his proposals, experimental work has shown the above requirements are met. A number of recent reviews have indicated a general acceptance of this statement (Racker, 1976; Capaldi, 1977; also see the series of papers in the *Ann. Rev. Biochem.* **46,** 1977). Striking progress has come from the laboratories of Racker, Hackenbrock, Capaldi and others on the vectorial arrangements of the protein components of the membrane. The statement applies equally well to the lipids and these membrane components will certainly emerge as an important part of the oxidative phosphorylation story as Knowles, Kandrach, Racker & Khorana (1975) have already shown.

If protons are driving ATP synthesis (Mitchell, 1974) then the proton is

necessarily transported from the *C*-side to the *M*-side of the mitochondrial membrane. It is further assumed by this hypothesis that electron transport is the source of energy for both the movement of the proton across the membrane and the synthesis of ATP.

One of the more surprising features of the mitochondrial membrane is the presence of a high concentration of polyunsaturated fatty acids (Guarneri & Johnson, 1970). It is surprising because, on the one hand, the doubly allylic protons on the methylenes between the double bonds are so very sensitive to oxidation by molecular oxygen. On the other hand, oxygen must pass through the membrane to the *M*-side so that cytochrome oxidase may pass on an electron to it and thereby complete the electron transport chain! Furthermore, Racker has found that reconstituted systems of cytochrome oxidase and the ATPase require polyunsaturated fatty acids for full activity (Racker, 1976).

The application of potential compaction to oxidative phosphorylation may be considered a molecular description of the chemiosmotic hypothesis of Mitchell. It is entirely consistent with Mitchell's hypothesis and offers an explanation for how the mechanisms proposed by Mitchell are achieved. In the proposed description of molecular events, the driving force is the creation of a membrane potential by the reduction of oxygen by cytochrome oxidase.

Let us say that the electron at reduced cytochrome *c* (on the *C*-side of the membrane) is passed through the membrane via the hemes of cytochrome oxidase and reduces oxygen. This event will immediately place a local potential on the membrane in which the *M*-side becomes negative and the *C*-side becomes positive. It is further proposed that the potential thus impressed locally on the membrane compacts the membrane at that point. This compaction is facilitated by the presence of polyunsaturated fatty acids in the vicinity of cytochrome oxidase. Through the medium of the phospholipids, which convey the compaction in the plane of the membrane, this compaction creates conformational changes in the transmembrane subunits of the ATPase (F_0). These conformational changes facilitate the migration of protons across the membrane from the *C*-side to the *M*-side. Protons are driven by the electrical field. They may be retained within the ATPase in a closed environment. The downhill energy transfer of a proton is coupled, as proposed by Mitchell (1961), to the synthesis of ATP. The existence of a residual potential across the membrane enhances the migration of protons in F_0. The significance of the cytochrome oxidase compaction is that it organizes the system and connects those transmembrane components of F_0 through which the protons migrate. The number of protons that migrate, according to this proposal, may vary according to the ΔpH. Since a high concentration of protons may itself create a potential and thereby a

compaction, and since thermal molecular motion may itself facilitate the conformational changes, these effects may contribute to proton migration in the absence of a cytochrome oxidase compaction.

The transfer of protons from the *C*-side to the *M*-side, however, also reduces the potential across the membrane. The release of the potential *expands the compacted membrane*. The expansion of the membrane likewise produces a conformational change in cytochrome oxidase. This change, it is further proposed, now arranges the protein in the appropriate transmembrane conformation for another electron to traverse the membrane.

A repetition of these events will produce a wave. Such a wave will be a standing wave of expansion and contraction, and as discussed previously, the mechanical wave is also associated with an oscillating electrical field. Such a standing wave would have regions of maximal vibrations (F_0 and cytochrome oxidase) and nodes. Because it is a lattice wave, one cannot relate the frequency to the wavelength through the velocity.

The electrical potential field around cytochrome *c* has been calculated (Koppenol, Vroohland & Braams, 1978) for the tuna and horse proteins. The results indicate that the protein has a dipole moment of about 240 D. The results also indicate that the dipole axes of both proteins are similarly oriented and indeed the positive end of the dipole emerges perpendicular to the heme. If cytochrome *c* were on the surface of a membrane with an oscillating field it would roll back and forth between two sites each time the field oscillated. These oscillations would be in unison with the turnover number of cytochrome oxidase.

The decline of free energy as the electrons pass through this third site (cyt *c* to oxygen) is just double that of the other two sites of electron transport. It would not be surprising therefore that the nature and the role of this site is different than that of sites I and II. The number of protons that traverse the membrane at each site is under active investigation (Brand & Lehninger, 1977) and many protons may be driven through the ATPase on a single compaction. It is interesting that the head of the ATPase (which contains the ATPase activity) is on a stalk. The stalk raises it about 7 nm from the surface of the membrane which implies that any dipoles in the active site(s) of the ATPase are not subject to the field oscillations within the membrane. This would be important if the *P*/O ratio were greater than one unless the *P*/O ratio were determined by the number of active sites on the enzyme, which does not seem to be the case (Racker, 1976).

(D) POTENTIAL COMPACTION AND TRANSPORT ACROSS BIOLOGICAL MEMBRANES

The Mitchell hypothesis in its initial formulation applies equally well to the energetics of transport of many substances across biological membranes.

In the case of *E. coli*, this has been convincingly shown by Kaback (1977), who was led to these conclusions by his data. In this system, the electrochemical gradient of protons is generated by D-lactate and lactate dehydrogenase. It is generated at a point in the respiratory chain before cytochrome b_1. The inside is negative and the outside is positive.

The bacterial system is devoid of polyunsaturated fatty acids, indeed the monounsaturated fatty acids may be replaced by cyclopropane groups or other kink producers. Following the hypothesis one would say that it was less efficient and had greater damping.

In *E. coli*, the requirement for unsaturated fatty acids for transport has been established by Wilson *et al.* (1970) and by Overath *et al.* (1970). It is interesting that *E. coli*, which has exclusively monounsaturated and cyclopropane fatty acids can transport effectively with a mixture of 9-bromo- and 10-bromostearic acid as a replacement of these fatty acids (Fox *et al.*, 1970). The bromo group, which is sterically about the size of an ethyl group, causes a kink in the chain at the correct position. These investigators could not replace the unsaturation requirement with 9,10-dibromostearic acid. This molecule does not have the appropriate kink because the second bromo group would assume a conformation *trans* to the first bromo and this would straighten out the chain. The inability of the dibromostearate to substitute for monosaturated fatty acids demonstrates that the bulkiness of the group in the plane of the membrane is not as significant as the single kink which is perpendicular to the membrane. It suggests that the function of the unsaturated fatty acids, which the monobromo acids successfully replaced, is primarily to facilitate compaction rather than lateral fluidity in the plane of the membrane.

The implication of the potential compaction hypothesis for *E. coli* transport is that the generation of a membrane potential by the electron transport particles of the membrane would drive a variety of transport proteins in a standing wave pattern. Each would collapse the electrochemical gradient by carrying through a proton to the negative interior.

(E) CODA

It is the nature of living cells that they consume energy in an unending exchange for molecular order. Nonetheless, the cell envelope exists in an environment of Brownian motion. This random motion must inevitably affect the conformations of transmembrane proteins with its impacts. One might assume that the transport of substances across membranes would depend upon the exquisite conformation of the proteins in the hydrophobic bilayer as it does with enzyme active sites. It seems likely that the cell either

utilizes this motion or it has developed a mechanism for preventing it from interfering with its molecular organization. The organized motion of the compaction waves herein proposed may serve to overcome the randomizing influence of Brownian motion so that the cell maintains control over the transport process. This activity would be at the expense of the energy required to maintain these waves. It is necessary that the magnitude of the energy level of the source of the compaction waves be greater than kT (thermal energy) which is the magnitude of the energy of Brownian motion.

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