ENERGY-TRANSDUCING REACTIONS IN BIOLOGICAL MEMBRANES

I. ENERGY TRANSDUCTION IN ION- AND ELECTRON-EXCHANGE POLYMERS

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Summary

Theoretical and experimental studies of the properties of rubbers, and of ion- and electron-exchange polymers, show that mechanical deformation, the application of heat, adsorbing solvent or solute molecules onto the polymer chains, or changing the crosslinking alters the free energy changes of interactions occurring at ion-exchange, electron-exchange, or other adsorption sites of the polymer as a result of changing a dilation–contractile equilibrium, and provides means for the interconversion of mechanical, heat, osmotic, and chemical energies. In all these processes polymer entropy changes participate in the overall free energy changes of the system.

Biological energy-transducing systems are envisaged as specialized polymeric ion-exchange membrane systems of fixed or variable crosslinking, and with ion-exchange sites which may or may not possess electron-exchange properties. Dilatory processes associated with the exchange sites in equilibrium with ion concentration gradients, or resulting from oxidation of electron-exchange sites, are opposed by elastic contractile processes associated with entropy changes within the polymeric matrix to which the exchange sites are attached. A state of equilibrium is established between the two opposing processes in which the overall free energy reflects the entropy changes of the polymeric matrix.

Contractile tendencies are postulated to arise from elastic deformation of polymeric lipid bilayers with rubber-like properties. Interaction of the bilayers with drugs or hormones changes the rotational freedom of polymer segments and so influences their elasticity. In more complex processes, an additional mechanism involving a reversible thioester crosslinking reaction with ATP, ADP, and P_i also alters the matrix elasticity, and provides a reversible mechanism for interaction between an ion-exchange reaction and a phosphorylation reaction.

The dilatory–contractile processes may result also in conformational changes which alter the specificity of adsorption sites and enable interactions between adsorbate and such sites to influence the dilatory–contractile equilibrium.

A phospholipoprotein with ion-exchange properties is described to illustrate how the lipids in such a structure could form a bilayer which would be expected to exhibit rubber-like properties, and how dilation could change it from a K^+-selective to a Na^+-selective structure.

I. Introduction

There is extensive knowledge, but little understanding at the molecular level, of the biological membrane processes associated with action potentials, the sodium pump, and mitochondrial oxidative phosphorylation and ion transport. This series of papers aims to interpret these processes at a molecular level and to show that they are but variations of a common theme in which an equilibrium is established between opposing dilatory and elastic contractile processes.

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The basic problems to which this series of papers is directed are as follows. What is the molecular mechanism which enables an excitatory current, odours, or light to induce an ion-permeability change in nerve membranes, and by what molecular process is it influenced by membrane stabilizers and labilizers? By what molecular mechanism does hydrolysis of ATP induce an ion-permeability change (and vectorial action) in a sodium pump which enables Na^+ to be actively transported in exchange for a smaller number of K^+ ions, and what is the molecular mechanism for inhibition of the sodium pump by cardiac glucosides and anaesthetics? What is the molecular process involved in intermediate energy conservation in mitochondrial oxidative phosphorylation? How is it related, at a molecular level, to oxidation–reduction reactions in the respiratory carriers, and to the processes of oxidative phosphorylation without formation of an intermediate high-energy phosphate, to ion transport, and to the pyridine nucleotide transhydrogenation reactions? What is the molecular mechanism of inhibition by uncoupling agents, oligomycin, and some anaesthetics? In all three energy-transducing processes lipid is important—why is this so? Are these processes interrelated?

A number of hypotheses have been proposed to account for these processes but these have usually been on a general basis without discussion at a molecular level. When a molecular description has been attempted it has been confined to part of the system without considering the whole, or its relationship with other energy-transducing processes.

Discussion has been limited to the above three major energy-transducing processes because the wealth of experimental data justifies some degree of speculative interpretation at a molecular level. Each exemplifies one major type of biological energy-transduction process so that understanding of these processes would establish principles which might be expected to have broader implications. In each, a lipoprotein membrane undergoes expansion–contraction cycles involving lipid and ions, and each is influenced by anaesthetics. These three processes will be interpreted at a molecular level in terms of ion-exchange processes of varying complexity and involving elastic polymeric matrices associated with lipid.

II. Dilatory–Contractile Membrane Processes and Energy Transduction

The biological energy-transducing systems are envisaged as specialized, polymeric, ion-exchange systems of fixed or variable crosslinking, and with ion-exchange sites which may or may not possess electron-exchange properties. Dilatory processes associated with the exchange sites are opposed by contractile processes associated with the polymeric matrix to which the exchange sites are attached. A state of equilibrium establishes between the two opposing processes. The dilatory–contractile process may result also in conformational changes which alter the specificity of adsorption sites so that interaction between adsorbate and such sites also influences the dilatory–contractile equilibrium.

Dilation arises from osmosis, and from electrostatic repulsive forces between adjacent ion-exchange sites, and reflects the interaction energies of ion concentration gradients with the exchange sites, or increasing charge and reduced electron-donating properties as the result of oxidation in the case of electron-exchange polymers. The opposing contractile processes are postulated to arise from the energy changes

associated with elastic deformation of the polymeric matrix by the dilatory processes. Deformation will be more difficult if the matrix is a "glass" rather than a "rubber", and will be determined by the extent of its crosslinking and the rotational freedom of its polymer segments.

In the simplest of the processes, that involved in action potentials, the exchange sites do not participate in electron exchange and the crosslinking is fixed. Elastic deformation is postulated to stretch and orientate polymeric lipid bilayers with rubber-like properties which develop a tension on dilation. Excitation promotes transient dilation by replacing Ca^{2+} with Na^{+}, and the dilation changes the ion selectivity of the exchange sites. Interaction of drugs, hormones, or odours with the membrane bilipids, or heating, changes their elasticity and influences membrane permeability by controlling the extent of dilation–contraction, an effect analogous to changing the crosslinking of an ion-exchange resin.

In the sodium pump, a similar membrane permeability system and elastic matrix is postulated to be involved as in action potentials, but has the additional complexity of a variable thioester crosslinking reaction involving ATP, ADP, and P_i. Increasing resistance to deformation of the polymeric matrix results from increasing crosslinking, so that the state of equilibrium of the ion-exchange process depends on that of the crosslinking reaction and *vice versa*. Such a mechanism therefore enables a phosphorylation reaction involving ATP to influence ion-exchange reactions and associated ion concentration gradients in a reversible manner. The equilibrium positions of the crosslinking and ion-exchange reactions may be influenced by changing the concentrations of any of the participants, and by other interactions which influence membrane elasticity such as those with some drugs, hormones, heating, or, in a specialized system, as a result of photoisomerization.

In the highly complex process of mitochondrial oxidative phosphorylation, oxidation–reduction reactions in electron-exchange polymers induce dilation–contractile changes which are considered to change membrane thioester crosslinking and which is interconnected by a specialized proton-transfer reaction to oxidative phosphorylation. Membrane elasticity is again an important factor.

All such processes have in common important entropy contributions from the polymeric matrix of the exchange sites to the overall free energy changes of the energy-transducing processes. The entropy changes relate to polymer elasticity which enables the free energy changes to be regulated by hormone interactions influencing the rotational freedom of polymer segments.

The remainder of this paper will establish an experimental foundation for the physicochemical principles involved, and subsequent papers will utilize them to interpret the biological data. This part should therefore be read, not in isolation, but in conjunction with the later parts.

III. Polymer Elasticity and the Rubber Bilayer Hypothesis

(*a*) *Some Polymer Properties*

Linear polymers can exist in three different states. At low temperatures the chains of amorphous polymers freeze into a glass-like, brittle state which cannot undergo extensive deformation without destruction; this is the "glass" state. At higher temperatures, or in the presence of small amounts of swelling solvents, the

chains acquire some mobility and enter a viscoelastic "rubber" state which can undergo substantial deformation without destruction. At temperatures above the flow temperature they pass into a viscofluid state. Crosslinking prevents flow so that crosslinked polymers can exist only as a glass or a rubber. Transition into the glass state is a kinetic, not a thermodynamic, phenomenon so that the glass temperature is a function of the rate of cooling (see Volkenstein 1963).

Stretching rubber releases heat and develops a crystalline structure as shown by X-ray diffraction studies. The crystallinity disappears when stretched rubber is heated or exposed to some solvent vapours (see Rogers 1953; Volkenstein 1963). Heating stretched rubber develops a tension which can perform mechanical work. Below a critical elongation, the "thermoelastic point", heating rubber expands it and the strain diminishes as in any other substance. Above the inversion point the rubber contracts, and the strain increases with increasing temperature; such behaviour uniquely characterizes a rubber.

In a relaxed, rubber-like state, linear polymers can assume many different conformations by virtue of the rotational freedom of their segments. On stretching, the chains come closer together, since the volume of the polymer does not alter significantly, and the rotational freedom of the segments diminishes. The classical theory of rubber elasticity attributes elasticity to the gain in entropy when stretched rubber contracts (see Volkenstein 1963). Earlier theories ignored changes in internal energy, which are small, and more recent work has established that intramolecular forces contribute more to the energy changes than do intermolecular forces (Shen, Hall, and De Wames 1968).

At a given temperature many polymers with the properties of a glass become rubbers in the presence of small amounts of swelling solvents. These bind to the polymer chains and, by decreasing interactions between the chains, increase the rotational freedom of the polymer segments. At much higher solvent concentrations the polymer dissolves in the solvent. At a fixed temperature, the elongation corresponding to the thermoelastic point of natural rubber changes with varying amounts of n-hexadecane (Oplatka and Katchalsky 1966). Solvents therefore have a profound influence on the thermodynamic and elastic properties of polymers; small amounts can enhance rubber-like elasticity but large amounts destroy it.

Because the elasticity of a polymer reflects the rotational freedom of its segments, the stereochemical properties of a polymer strongly influence its rubber-like behaviour. For example, natural rubber is a *cis*-polyisoprenoid but the *trans* isomer, gutta percha, only becomes a rubber at temperatures above about 70°C, since free rotation of the isoprene units is hindered by the methyl substituents.

(*b*) *The Rubber Bilipid Hypothesis*

It is postulated that at least some of the lipids associated with biological energy-transducing properties of membranes are present as bilayers in which the polar portion of the lipids are *attached* to supporting structures and the hydrocarbon structures intermingle (Fig. 1). When relaxed, the hydrocarbons are in a liquid-like state with high rotational freedom for segments of the chains. If a transverse stretching force is applied to the supporting structures, the chain mobility diminishes

as the structure extends and the chains come nearer together. The situation therefore resembles that of a rubber and a tension develops in the stretched structure. From such a viewpoint the polar structures of the lipids become important, not because they impart surface activity, but because they provide sites for attaching hydrocarbon structures to proteins and other polymers. Mechanical attachment is essential for developing a tension. In the light of such an hypothesis, phosphatidylserine acquires particular significance because it can form lipopeptides by polymerization of its serine groups. More complex lipids, e.g. gangliosides, may also be involved in polymer structures.

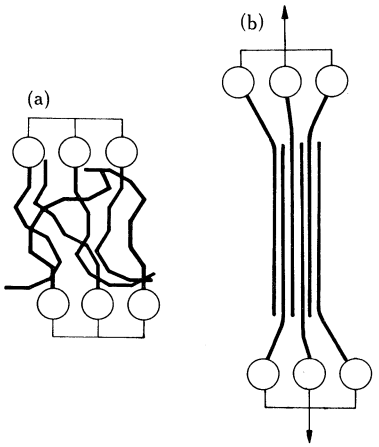


Fig. 1.—When bilipids are attached at each end to supporting structures (*a*) stretching them develops a tension, as in rubber, because of increased ordering of the hydrocarbons and decreased rotational freedom of hydrocarbon segments (*b*).

Penetration of the hydrocarbon phase of such a lipid bilayer by foreign substances can have two types of effects. Small amounts of some substances will swell the hydrocarbon phase, and increase the mobility and elasticity of the hydrocarbon chains; this is the function of membrane "stabilizers" such as anaesthetics. Large amounts of the same substances will "dissolve" the hydrocarbons and rupture the membrane ("lysis"). There will therefore be a critical concentration of such stabilizers, for a given temperature, at which membrane elasticity is a maximum. A maximum in membrane stability as a function of stabilizer concentration has been observed with many biological membranes (see Seeman 1966) and will be considered further in Part II of this series (Weiss 1969*a*).

Other substances will interact with the polar groups and the lipid hydrocarbons to form molecular complexes such that the hydrocarbon chain movements freeze into a glass. Some steroids, e.g. cholesterol, and drugs exhibit such properties (see Part II).

If the rubber bilipid hypothesis be valid there should be evidence that relaxed hydrocarbon lipid structures in appropriate membranes are in a liquid-like state which becomes more ordered on dilation of the membrane, that lipids associated with energy transduction show stereochemical effects, and that they are more likely to be unsaturated than saturated. There is substantial support for such conclusions.

Spin-labelling studies establish the existence of liquid-like hydrophobic regions in some excitable membranes and synthetic phospholipid bilayers. The extent of the fluid regions increases on addition of some thiols, hydrocarbons, or tetracaine, but

decreases on the addition of cholesterol or gramicidin (Hubbell and McConnell 1968). At 37°C, which is above the largest endothermic transition, the hydrocarbon chains of some pure lipids are in a liquid-like state (Chapman, Byrne, and Shipley 1966), a property which recalls the liquid-expanded state of some monolayers.

Light scattering and birefringence changes at the peak of an action potential show expansion of the membrane and greater ordering (Cohen, Keynes, and Hille 1968; Tasaki *et al.* 1968).

Many important membrane lipids are unsaturated and exhibit stereochemical specificity. Unsaturation and changing from *trans* to *cis* configurations depress the transition temperatures of lipids (Chapman, Byrne, and Shipley 1966). Mitochondrial membranes contain large amounts of unsaturated lipids with up to six double bonds which are essential for oxidative phosphorylation; their function seems to be a structural one (Fleischer *et al.* 1962; Fleischer and Rouser 1965). Photosynthetic membranes require α-linolenate (Erwin and Block 1964). Mammals have an essential dietary requirement for linoleic acid which must be *cis-cis*, from which they synthesize longer chains with more unsaturation (Levin, Johnson, and Albert 1957; Fleischer *et al.* 1962). The ubiquinones, which are essential components in oxidative phosphorylation, have isoprenoid groups for side chains (see West *et al.* 1966) and vitamin A seems to be important for membrane stability (see Seeman 1966).

There is therefore some support for postulating an elastic membrane component composed of lipid as an essential element in biological energy-transducing membranes. The next section will discuss the influence of crosslinking, which determines the extent of configurational polymer changes, on the ion selectivity of ion-exchange systems with fixed crosslinking.

IV. Ion-exchange Systems with Fixed Crosslinking

(*a*) *Properties of Crosslinked Polyelectrolytes*

Increasing the crosslinking of an ion-exchange resin increases its ion selectivity. Several theories account for the phenomenon from different points of view (Helfferich 1962).

A quantitative theory has been developed by Rice and Nagasawa (1961). In this theory, the exchangeable ions of an ion-exchange resin of the strong-electrolyte type are considered to be held either dissociated from the exchange sites in an ion atmosphere, or in an undissociated state as ion-association complexes with higher interaction energies. Ion selectivity is determined by the extent of ion association which is related to the concentration and charge density of the exchange sites, to the distance between the charges, and to the effective dielectric constant of the medium in the immediate vicinity of the fixed charges. Electrostatic repulsive interactions between the exchange sites arise mainly from the dissociated sites so that the extent of the interaction decreases with increasing ion association and separation of the sites. The electrostatic repulsive forces and osmotic effects associated with the charges tend to dilate the structure to an extent which is determined by the degree of crosslinking of the polymer. Increasing crosslinking decreases the distance between the fixed charges of the dilated polymer and the resulting higher electrostatic potential is minimized by ion association. Consequentially the selectivity of the resin increases with the crosslinking.

Analogous principles would apply, but have not yet been computed, when the fixed charges have the properties of a weak electrolyte. Such resins can relieve an electrostatic potential by forming undissociated sites as a result of accepting or losing protons, according to whether the sites are acidic or basic, as well as by ion association. Altering the crosslinking changes the acidity of weak-acid ion-exchange resins (see Weiss *et al.* 1966).

The thermodynamic contributions of the polymer configurational changes to the overall free energy change of an ion-exchange reaction depend also on whether the polymer matrix is a glass or a rubber. The subject has received little study in the ion-exchange field, but recent work by Boyd and Larson (1967) suggests that a selectivity reversal observed on reducing the crosslinking of an ion-exchange resin may be due to a transition from a glass to a rubber.

It is therefore well established that changing the crosslinking of an ion-exchange resin changes the thermodynamics of reactions associated with its exchange sites by altering its dilation tendencies. Since crosslinking changes polymer elasticity, it follows that polymer interactions with adsorbates which also change its elasticity will likewise influence the ion-exchange reactions.

(*b*) *Energy Conversion in Crosslinked Polyelectrolyte Systems*

The degree of dilation of a weak-electrolyte resin can be increased by increasing the dissociation of the fixed charges either by changing the pH or by reducing the ionic strength of the contacting solution. Such effects can be utilized for performing mechanical work in model muscles (Kuhn *et al.* 1960), in mechanicochemical engines (Steinberg, Oplatka, and Katchalsky 1966), or to produce an action potential in a model membrane (Walters, Kuhn, and Kuhn 1961). Desalination in an ion-exchange resin of the strong-electrolyte type has been achieved by applying pressure (McKelvey, Speigler, and Wylie 1957). Mechanical deformation of a weak-electrolyte resin also changes the pH value of a solution in equilibrium with it (Kuhn *et al.* 1960). Heating a mixture of weak-acid and weak-base resins reduces their dissociation and is the basis of a new approach to desalination (Weiss *et al.* 1966).

Similar principles apply to electron-exchange resins where oxidation or reduction changes the degree of ionization of the resin and the resultant osmotic forces and electrostatic interactions induce contraction or expansion. Kuhn *et al.* (1960) have shown that mechanical deformation of such a system leads to a change in its oxidation–reduction potential.

These theoretical and experimental studies, together with those of rubbers, have established that mechanical deformation, the application of heat, the interaction of solvent or solute molecules with the polymer chains, or changing the crosslinking alters the free energy changes of interactions occurring at ion-exchange, electron-exchange, or adsorption sites of a polymer and changes its dilation–contractile equilibrium, so providing means for the interconversion of mechanical, heat, osmotic, and chemical energies.

(*c*) *Ion Selectivity*

A high interaction energy, characteristic of ion association at an ion-exchange site, reduces the electrostatic potential of an ion and, as noted above, increases ion selectivity of the exchange reaction according to the theory of Rice and Nagasawa

(1961). Ling (1962) and Eisenman, Sandblom, and Walker (1967) have emphasized also the importance of ion association, hydration energies, and the size of cavities adjacent to the exchange sites in determining ion selectivity in biological systems. All the simple ion-exchange polymers so far considered involve hydrated ions, but over the last decade substantial evidence has accumulated showing that some or all of the water of hydration of alkali metal cations can be replaced with other ligands involving ion-dipole interactions with the cations.

A hydrocarbon solution of di(2-ethylhexyl)phosphate extracts Na^+ in preference to K^+ from a buffered aqueous phase (see Keder, Martin, and Bray 1965). At low degrees of dissociation of the acid, the Na^+ in the hydrocarbon is solvated by the undissociated acid; the Na^+ is hydrated in the hydrocarbon when the acid is highly dissociated (McDowell and Coleman 1965). If a sterically hindered phenol is added, particularly 4-s-butyl(α-methylbenzyl)phenol (BAMBP), the extraction selectivity order is reversed, and K^+ is extracted into the oil phase in marked preference to Na^+ (Bray 1964; Keder, Martin, and Bray 1965; Zingaro and Coleman 1967). These studies have shown that the extracted cations are solvated by four undissociated molecules of BAMBP and that their charge is neutralized by the anions of the phosphate diesters. The normal affinity series based on hydrated radii is reversed because it is easier for BAMBP to displace the water of hydration from the larger than from the smaller cations when reckoned on the basis of their unhydrated radii.

A number of macrocyclic antibiotics, such as nonactin, monactin, the enniatins, and valinomycin, show a high degree of cation specificity. A recent X-ray study of the nonactin–K^+ complex shows that K^+ is surrounded by four ether-oxygen atoms from furan rings and by four keto-oxygen atoms (Kilbourne *et al.* 1967). Such macrocyclic antibiotics readily form highly selective lipid-soluble complexes with various alkali metal ions in a lipid membrane (Eisenman, Ciani, and Szabo 1968; Pressman 1968). For example, valinomycin shows a marked selectivity for K^+ rather than Na^+. Pedersen (1968) has synthesized a range of macrocyclic polyethers in which ether-oxygen atoms solvate alkali metal cations. It is probable that a number of lipopeptides will accommodate K^+ in preference to Na^+ within a lipid membrane in which the solvating groups are the keto-oxygen atoms of amide or ester groups, ether-oxygen atoms in some lipids, and vinyl ether-oxygen atoms in the case of plasmalogens.

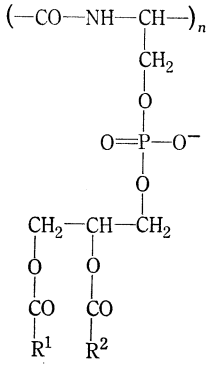
The selectivity of such interactions would be expected to change with the stereochemistry of the peptide. The ion-specificity of valinomycin, for example, is highly sensitive to steric changes, but the substitution of the L-lactic acid residue by L-alanine does not change its activity (Shemyakin *et al.* 1965). Blondin and Green (1967) have claimed that the ability of poly-L-lysine to complex alkali metal cations increases with the helical content of the polymer and shows a selectivity in the order $Rb^+ > K^+ > Na^+$, which is the order observed in many biological membranes.

It is therefore reasonable to postulate that there exist within biological membranes peptides, or more probably lipopeptides, which confer cation selectivity to the membrane according to their configuration, and bind alkali metals in ion-association complexes in a partly or completely unhydrated state.

In order to illustrate how the above principles might apply in a biological system, the next section will describe a remarkable lipoprotein and will consider properties that might be expected of it.

V. A Biological Ion-exchange Polymer with Fixed Crosslinking

There is present in nervous tissue a phospholipoprotein discovered by Heald (1961*a*, 1961*b*, 1962), who observed its ion-exchange properties and suggested that they might be involved in cation transport. The phospholipoprotein contains a high proportion of glycerol esters and up to six consecutive *O*-phosphoseryl residues, which are probably attached to lipids as follows:



where $n = 4$–6 and R^1 and R^2 represent lipid hydrocarbons.

Such a phospholipoprotein might assume a helical, polypeptide configuration from which the lipids radiate and are attached by Van der Waal interactions with adjacent lipid components of a membrane to form a molecular "spring" with cation-permeable regions and lipid bilayers. Ionization of the phosphate groups would create electrostatic repulsive forces which, together with osmosis, would tend to expand the spring to an extent depending on the degree of interaction between the cations of the solution and the spring anions. The dilation stretches the attached lipid bilayers which, becoming more ordered, develop an elastic contractile tension as in rubber. It is well known that the helical content of polypeptides increases with ionic strength and that it decreases with increasing ionization. Small helices are inherently unstable because of "end-effects", but the lipid could be expected to stabilize the structure if it were attached to the membrane.

In an expanded state, the size of cavities containing the exchange sites, and the charge density of the latter, might be such that of the alkali metals only hydrated Na^+ (or Li^+) can be easily accommodated. The situation would be somewhat analogous to the selective extraction of Na^+ by di(2-ethylhexyl)phosphate in kerosene discussed above.

In a contracted helical form, the cavities adjacent to the exchange sites are small and, as can be shown with a model, have dimensions which might enable them to accommodate unhydrated but not hydrated alkali metal cations. Unhydrated ions thus accommodated could then be solvated mainly by a cage of neighbouring oxygen atoms from the carbonyl groups of the ester (or ether) and amide structures in the spring, and which are favourably orientated for such a purpose when the spring is contracted but not when it is expanded. K^+ would then be accommodated in preference to Na^+ because of its smaller hydration energy. In such a situation particularly strong electrostatic interactions would occur, due to the low dielectric

constant of the medium adjacent to the exchange sites, and would promote extensive ion association. Such a situation therefore resembles that of K^+ in cyclic peptides, such as valinomycin, and the increasing selectivity shown by poly-L-lysine for K^+ as its helical content increases.

The magnitude of the elastic tension which develops on dilation of the spring depends on the rubber-like properties of the bilipids. Interaction of foreign substances with the bilipids will alter their elasticity and the relative stabilities of the dilated and contracted structures. Anaesthetics are postulated to increase chain mobility; the enhanced elasticity stabilizes the contracted K^+ structure. Such effects, and others of opposite kind, will be considered in Part II in connection with action potentials.

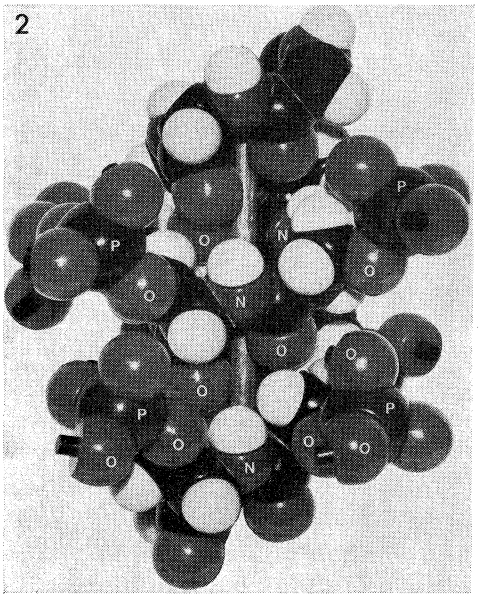
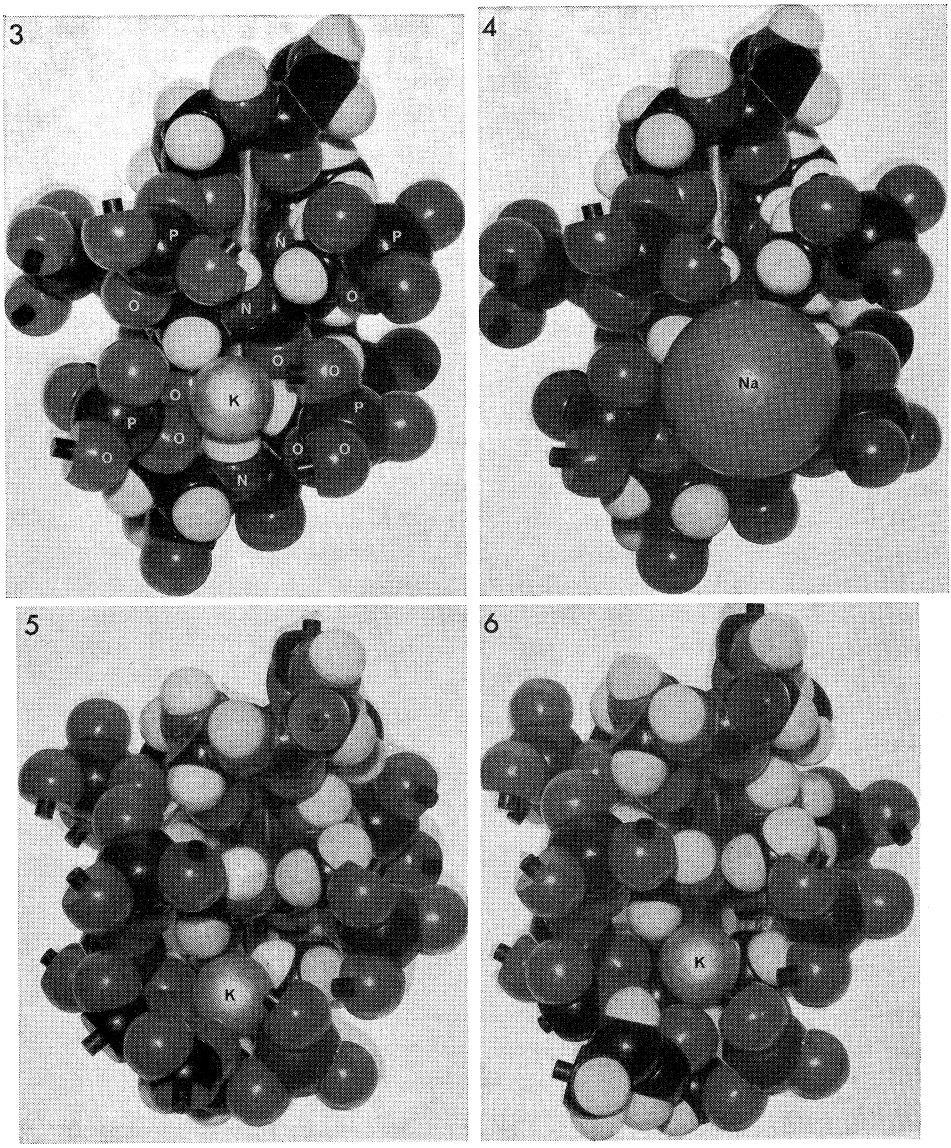


Fig. 2.—Model of the "spring"—a helical polypeptide containing six consecutive phosphatidylserine residues.

Figure 2 shows a model of a helical peptide containing six consecutive seryl-phosphate residues. Two additional peptide linkages are also present; for clarity, the lipids have not been attached to the phosphate groups. The inner pair of peptide carbonyl groups is available for reaction with K^+ held within the spring. Figure 3 shows one unhydrated K^+ in place. Figure 4 shows, for comparison, hydrated Na^+: this group cannot get close to the peptide chain even without the lipid in place; with the lipid in position access becomes even more difficult. Figure 5 shows portion of a diglyceride unit, devoid of the hydrocarbon portions, attached to one phosphate group; unhydrated K^+ still fits into the spring cavity but is now close to the carbonyl groups of the lipid esters as well as those of the polypeptide. Figure 6 shows portion of a plasmalogen lipid attached instead of the diglyceride; in this situation the highly polarizable vinyl-ether-oxygen atom is close to the K^+.

These models establish the feasibility of the proposal and show in terms of one specific structure how a conformational change might change Na^+: K^+ selectivity and how this could be influenced by changing the elasticity of a lipid bilayer. Later papers

in the series will refer to this model of Heald's lipophosphoprotein simply as the model "spring" and will show how it might account for the permeability changes associated with an action potential (Part II) and with the sodium pump (Part III, Weiss 1969*b*).



Figs. 3–6.—Models of the spring with alkali metal cations in place. *3*, Unhydrated K^+ in one position. *4*, Hydrated Na^+ in one position. *5*, Unhydrated K^+ and portion of a diglyceride lipid in position. *6*, Unhydrated K^+ and portion of a plasmalogen lipid in position.

The essential feature established by the models is that the carbonyl groups of the ester as well as those of the peptide can assume at least one configuration where they can be adjacent to K^+. There are probably other configurations where this is possible

and one in which the peptide is not helical may be preferable in view of the reluctance of polyserine to form helices. A further requirement is the necessity for a continuous channel connecting the exchange sites through the membrane, but insufficient model atoms were available to check this.

As noted above, the charge density of the exchange site is also an important factor determining the ion selectivity of an ion-exchange structure (see Reichenberg, 1966). A possible role for charge-transfer complexes in controlling ion selectivity by regulating the charge density of ion-exchange sites will be considered next.

VI. Charge-transfer Interactions in Biological Semiconductors

In Section IV it was shown how electrostatic interactions within ion-exchange resins influence the ion affinity and dissociation of the exchange sites. This section will discuss a second type of electrostatic interaction which can occur by the movement of mobile electrons, with the creation of a space charge, when an ion-exchange polymer has the additional property of being a semiconductor.

The semiconductivity of some proteins is attributed to the high polarizability of their polypeptide backbones. Electron donors, such as water or ammonia, induce n-type properties, whilst a variety of electron acceptors induce p-type conductivity in some proteins (Eley 1962; Snart 1968). Such effects arise from a change in the potential energy of the electrons (Fermi level) of the semiconductor due to a space charge resulting from the formation of a charge-transfer complex, a process which is analogous to chemisorption on inorganic semiconductors. Szent-Györgyi (1960, 1967) has shown that a wide variety of biologically active compounds can form strong charge-transfer complexes.

Recent studies have shown also that, as groups attached to semiconducting polymers are progressively ionized, further ionization of the remaining groups becomes increasingly difficult and ceases when only a fraction of the groups have ionized (Macpherson *et al.* 1965). The effect has been attributed also to the creation of a space charge within the semiconductor as ionization proceeds. Such an effect is illustrated in Figure 7. Analogous phenomena occur in activated carbons where the progressive reduction of quinone structures, or oxidation to carbonium ions, influences, and eventually prevents, further reaction (Weiss and Bolto 1965).

It is therefore possible that there may be some biological systems in which the formation of a charge-transfer complex between a hormone or a drug and the polypeptide backbone of a lipoprotein could polarize the polypeptide and thereby change the charge density and the ion specificity of a nearby ion-exchange site attached to the polarized structure. Such a concept resembles the idea of "cardinal sites" in proteins proposed by Ling (1962).

An alternative mechanism for achieving a similar result would be the formation of a charge-transfer complex with the vinyl group of a plasmalogen whose vinyl ether-oxygen atom is solvating a metal cation within a spring. Changing the electron density of the ether oxygen by formation of the complex would influence spring selectivity.

Having considered the likely properties of a simple type of biological ion-exchange polymer with fixed crosslinking, in which lipid elasticity can be altered only

by interaction of the lipid with foreign substances, in the next section a more complex situation is discussed where membrane elasticity can still be varied by adsorption of foreign substances but where much larger changes in elasticity are induced by changing the crosslinking of the membrane.

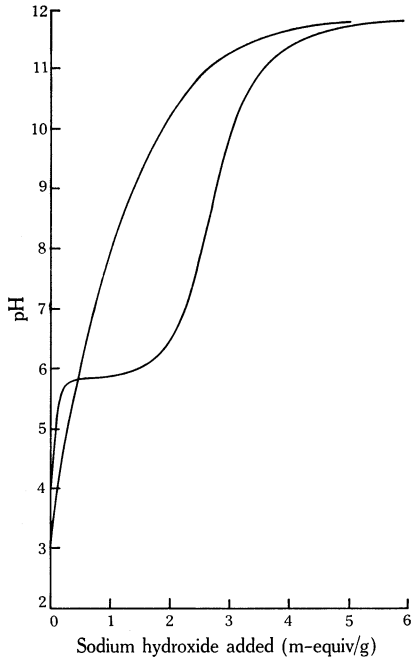


Fig. 7.—Titration curves of phthalic anhydride (lower curve) and of polymeric, semiconducting phthalic anhydride (upper curve). The steep rise in the titration curve of the semiconductor indicates that as ionization proceeds further ionization becomes progressively more difficult. The effect is attributed to space-charge interactions.

VII. Ion-exchange Systems with Variable Crosslinking

The properties will now be considered of an hypothetical ion-exchange polymer, such as the spring, but which can be crosslinked to a variable extent by crosslinks formed in a reversible chemical reaction. In such a system, a change in electrostatic potential arising from a reaction at the exchange sites can be accommodated by a change in the degree of crosslinking as well as by changing dissociation of the exchange sites. Osmosis, and electrostatic repulsive interactions between adjacent charges, tend to dilate the structure and are opposed by a reversible, chemically driven crosslinking mechanism which tends to contract the system. A position of equilibrium will be established where the two opposing forces are balanced. Such a system enables the free energy changes associated with reactions at the exchange sites to interact reversibly with those of the crosslinking reaction. Different ions will competitively inhibit, or stimulate, the crosslinking and associated reactions according to their interaction energies with the exchange sites.

A recurrent theme in subsequent papers in this series will be the concept of reversible crosslinking by the formation of a thioester between a carboxyl group RCOOH on one polymer segment and a thiol R'SH on a second polymer segment of a membrane:

$$RCOO^- + R'SH \rightleftharpoons RCOSR' + OH^-. \quad (1)$$

In some systems, such as the sodium pump for example, ATP will be considered to form an acyl phosphate which in turn provides energy for the thioacylation crosslinking reaction against an opposing reaction at the exchange sites (see Part III). The energy from hydrolysis of ATP thereby performs work at the exchange sites by forming the crosslink:

$$ATP^{4-}+RCOO^{-} \rightleftharpoons RCOOP_i^{2-}+ADP^{3-}, \tag{2}$$

$$RCOOP_i^{2-}+R'SH \rightleftharpoons RCOSR'+P_i^{2-}. \tag{3}$$

In other systems, an energized, externally applied contraction of the polymer is considered to synthesize the crosslink. Subsequent expansion of the polymer facilitates hydrolysis of the thioester and releases energy for the phosphorylation of ADP by P_i. In oxidative phosphorylation in mitochondria, the initial postulated contraction is provided by substrate reduction of the respiratory carriers (see Part IV, Weiss 1969*c*); in a reversed operation of the sodium pump, contraction is considered to result from a restriction of the ionic atmosphere at the exchange sites in the presence of a solution of high ionic strength (see Part III).

A somewhat related thioacylation reaction occurs in glyceraldehyde-3-phosphate dehydrogenase (Colowick, Eys, and Park 1966). Reactions (2) and (3) are analogous also to those which occur in anaerobic bacteria where ATP is synthesized in a reversible manner from ADP, P_i, and acetyl-CoA. The ΔF^0 of this reaction is zero (Stadtman 1966). The postulated concept of variable crosslinking with thioester crosslinks requires similar reactions to occur with the participating groups attached to polymeric chains. Under such circumstances, the ΔF^0 of the combined reactions (2) and (3) will no longer be zero, but it is reasonable to postulate that with a favourable polymer structure the ΔF^0 would remain within the range feasible for the participation of ATP as the energy source. It may be expected that the most suitable polymer would be selected in the evolutionary process.

Crosslinking reactions need not be confined to thioesters; indeed, in later parts the evidence for a thiol which could form a thioester is not usually equivocal and could apply also to histidine. Other structures may also provide a similar crosslinking function. However, for simplicity and clarity of presentation, the concept of thioacylation will be used where justified to establish a principle, even though alternative interpretations may be possible.

VIII. Ion Association and pH Sensitivity

The utilization of energy released during hydrolysis of a postulated thioester crosslink for the endothermic reaction of ADP with P_i can proceed by two mechanisms. In one it can occur, as in substrate phosphorylation, by a direct reaction between the participants with the formation of an acyl phosphate intermediate. There is evidence, to be discussed in Part III, that such a mechanism probably occurs in the sodium pump under reversed conditions. A second mechanism is possible, which does not involve an acyl phosphate intermediate and which will be postulated to be associated with processes of oxidative phosphorylation (Part IV). This mechanism involves a transfer of protons between a thioester and the enzyme catalysing a

reaction between ADP and P_i. Williams (1961, 1962) has suggested that analogous proton-coupling reactions could occur with advantage *within* a lipid membrane.

The phosphorylation of ADP by P_i may be written as follows:

$$ADP^{3-} + P_i^{2-} \rightleftharpoons ATP^{4-} + OH^-. \quad (4)$$

A study by Rutman and George (1961) of the influence of pH on reaction (4) shows that at pH 7·5 much of the change in free energy which occurs during the hydrolysis of ATP is contributed by the hydrogen ion term; at pH 7·0 it contributes about 50%. A comparison of reaction (1) with reaction (4) shows that, if the two reactions were coupled, hydrolysis of a thioester might drive the phosphorylation of ADP by P_i by removing the ensuing alkalinity. Such a proton transfer reaction resembles in one sense that between a carboxyl and amine ion-exchange resin where it has been demonstrated that extensive proton transfer requires that the ionized participants of the reaction form strong ion-association complexes with their counter ions so as to induce strong ion buffering (Weiss *et al.* 1966; Bolto *et al.* 1968).

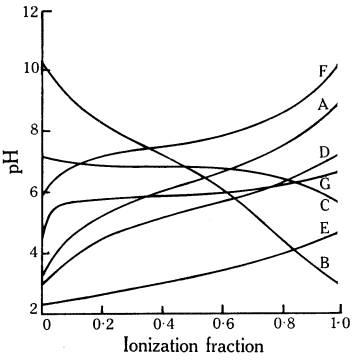


Fig. 8.—Titration curves of some crosslinked weak-electrolyte resins in 0·03M NaCl: *A*, poly(acrylic acid); *B*, polyvinylamine; *C*, poly(*N*-t-butylaminoethylmethacrylate); *D*, poly(acrylic acid) in 1M NaCl titrated with NaOH; *E*, as in *D* but titrated with $LaCl_3$; *F*, poly(methacrylic acid)—methylmethacrylate copolymer; *G*, di(2-ethylhexyl)phosphate in kerosene.

Theoretical studies of the titration of a polymeric acid RH with a base BOH usually assume the presence in the polymer of only the two species RH and R^-, since the cation B^+ is assumed to be held in a diffuse ionic atmosphere within the polymer. Theory then predicts a sloping titration curve for the acid (Michaeli and Katchalsky 1957). Such titration curves are observed with some ion-exchange resins (curves *A* and *B*, Fig. 8; Bolto *et al.* 1968). Hamann and Johnson (1968) have shown that if a second equilibrium exists between the dissociated salt R^-B^+ and a second species RB, in which B^+ is more strongly bound than in R^-B^+, then the titration curve becomes flatter as the equilibrium favours the formation of RB in the reaction $R^- + B^+ \rightleftharpoons RB$. The rapid rise in pH of the initial portion of the titration curve also diminishes, and eventually disappears, as the equilibrium increasingly favours the formation of RB.

Figure 9 shows the effect of increasing ion association in a weakly acidic dibasic acid. It is clear that ion association has the effect of flattening the titration curve to a remarkable extent.

Experimental studies with a series of homofunctional ion-exchange polymers by Bolto *et al.* (1968) and Weiss *et al.* (1966) have shown that flattening of the titration curves of weak-electrolyte resins occurs when the polarity of the exchange site becomes sufficiently low (curves *C* and *F*, Fig. 8), and thereby favours ion association, or when

the interaction energy increases with increasing charge on the ions (curve *E*, Fig. 8). A solution of di(2-ethylhexyl)phosphate in kerosene exhibits a particularly flat titration curve (curve *G*, Fig. 8; McDowell and Coleman 1965).

These, and other results, establish the validity of the theory of Hamann and Johnson (1968) and show that ion association, or interactions with multivalent ions, can increase greatly the sensitivity to pH changes of weak-electrolyte exchange sites and that such effects occur in an environment of low polarity. Such ion-association reactions in lipid membranes would induce strong pH buffering and would permit extensive interchange of protons between two such systems with only small effects on the pH of the medium. The thioacylation and phosphorylation reactions [(1) and (4)] are postulated to occur within a biological lipid environment in order to promote ion association which will be more pronounced if the cations are not hydrated heavily.

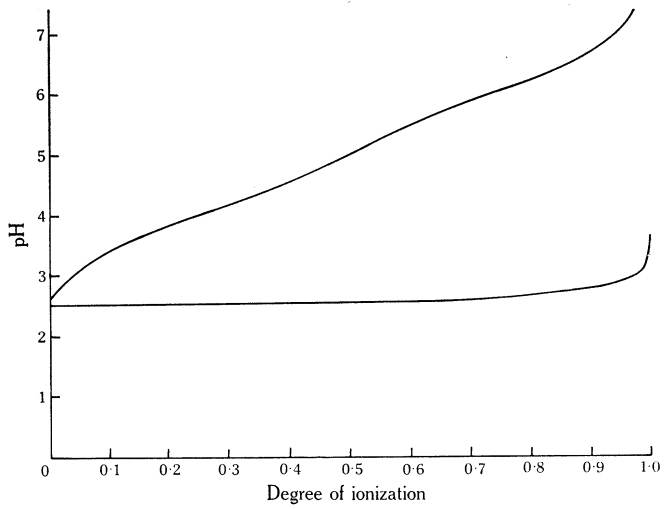


Fig. 9.—The effect of ion association on the titration curve of a dibasic acid. In the upper curve, no ion association is assumed. In the lower curve, strong ion association has been assumed. The calculations show that strong ion association results in a marked increase in the pH buffering capacity of the acid.

IX. Conclusions

It is concluded that there is a good physicochemical foundation for the hypothesis that biological energy transduction involves opposing dilatory and contractile processes which establish a reversible equilibrium and permit interaction between the separate equilibrium reactions participating in each process. The dilatory–contractile equilibrium can be regulated by additional interactions between a variety of drugs and hormones and polymer segments which alter polymer elasticity. There are biological observations supporting the concept that membrane elasticity may arise from attachment to supporting structures of lipid bilayers with rubber-like properties. The dilatory–contractile process may result also in conformational changes which alter the specificity of adsorption sites so that interaction between adsorbates and such sites influence the dilatory–contractile equilibrium and vice versa.

It has been shown also how a phospholipoprotein spring has a structure which, in the light of the general principles discussed above, can be expected to change on dilation from a K^+-selective to a Na^+-selective configuration. The spring has also attached lipid hydrocarbons which would enable it to attach to other lipid hydro-

carbons in a supporting membrane, and in so doing would provide a basis for an elastic retractile force opposing dilation. Part II will show how an excitatory current might induce dilation of such a membrane spring, and initiate an action potential, and how interaction of the lipid with drugs might control the membrane selectivity by influencing spring elasticity. Such a model then provides one specific example of the general principles discussed in this paper.

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