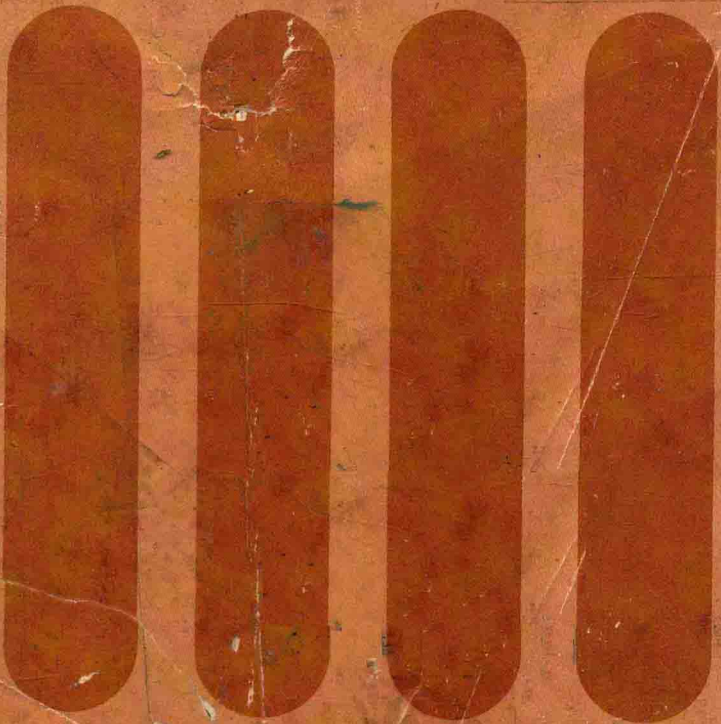
Advances in General and Cellular Pharmacology

VOLUME 2



Edited by T. Narahashi and C. P. Bianchi

Advances in General and Cellular Pharmacology

VOLUME 2

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PLENUM PRESS · NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Main entry under title:

Advances in general and cellular pharmacology.

Includes bibliographies and index.
1. Pharmacology–Collected works. 2. Cells, Effect of drugs on–Collected works. I. Narahashi, Toshio. II. Bianchi, Carmine Paul, 1927- [DNLM: 1. Pharmacology–Periodicals. 2. Cells–Drug effects–Periodicals. W1 AD612]
RM300.A38 615.'1 75-38640
ISBN 0-306-35072-6 (v. 2)

A Division of Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

Printed in the United States of America

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Preface

Advances in our understanding of the control mechanisms of cell function and cell-membrane properties have provided the general pharmacologist with better means of understanding drug action in cells. In the second volume of *Advances in General and Cellular Pharmacology*, the first chapter, by Drs. Lakshminarayanaiah and Bianchi, deals primarily with the physical–chemical action of ions and amphiphilic drugs with biological membranes. The second chapter, by Dr. Weiss, is concerned with the effects of drugs on cell calcium and contractility of vascular smooth muscle. Dr. Fassina's chapter on white adipose tissue emphasizes the importance of second messengers and energy-feedback loops in control systems as sites of action of drugs.

The ultimate goal of the pharmacologist is to use drugs to exert specific effects in modifying cell function to correct, prevent, or obviate disease or pathological processes. It is the purpose of these chapters to provide the reader with the interdisciplinary insight necessary to understand drug action at a cellular level.

C. Paul Bianchi
Toshio Narahashi

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1

Membranes, Ions, and Drugs

N. LAKSHMINARAYANAIAH AND
C. PAUL BIANCHI

The cells, particularly of animal tissues, are multiphase systems; but for purposes of consideration of material flow in them, they may be treated approximately as a three-phase system represented by

cell interior/cell boundary/cell exterior

Nearly 80% of the cell is made up of water. The major component of the cell interior is the potassium ion, whereas the major component of the cell exterior is the sodium ion. The chief intracellular anions are organic ions with a small amount of chloride ions which, however, are present in large quantities in the extracellular phase of the cell.

The cell boundary layer or the membrane is an important part of the cell, whose functions depend on the exchanges that occur between the cell and its environment. These exchanges take place across the membrane, which is freely permeable to water and some small ions (e.g., K^+ and Cl^-) and relatively impermeable to other ions (e.g., Ca^{2+}, SO_4^{2-}) and colloids. The integrity and state of the membrane are very important to the fate of the cell. Therefore a number of chemical substances (drugs) that act on the membrane can regulate the structure and function of the cell. The

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molecular basis of membrane–drug action can be followed only when the interactions of various components composing the system (membranes, ions, and drugs in water) are understood. Consequently, an attempt is made in this article to emphasize such ideas, real and/or imaginary, concerning the behavior of some biological and other model membranes subject to influences of variables such as ions and drugs.

I. MEMBRANES

Normally membranes are lipoprotein structures. Lipids and proteins are common to all membrane preparations (Dowben, 1969). Lipids account for 20–50% of the dry weight of membrane preparation. Of these lipids, free cholesterol accounts for 20–30% of the lipids, except in mitochondrial membranes which contain little cholesterol. The remaining lipids are mostly phospholipids (zwitterionic phospholipids such as phosphatidylcholine and sphingophosopholipids which have a net negative charge at physiological pH), and these are known to interact with the cholesterol, as revealed by the evaluation of area per molecule of mixed monolayers of cholesterol and phospholipids (area per molecule $<$ area per molecule of cholesterol plus area per molecule of phospholipid).

The lipids contain a variety of fatty acid residues ranging in carbon chain length from 16 to 22 carbon atoms and in the degree of unsaturation from 0 to 4 double bonds. The properties of phospholipids are governed by the nature of these fatty acid chains, a greater degree of saturation leading to closer packing and thereby increasing the chances for the prevalence of weak intermolecular forces. Longer fatty acid chains also increase the chances for the existence of short range London–van der Waals attractive forces. As opposed to this information about membrane lipids, not much is available about well-characterized proteins of the biomembranes.

Application of electron microscopy to the study of cell membrane structure appears to support the view already held on theoretical grounds that the living cell membrane is a bimolecular leaflet of lipid (50–70 Å thick) whose polar groups oriented towards the intra- and extracellular aqueous phases of the cell are lined with protein. This unit membrane structure was proposed by Danielli and Davson (1935). In various modifications it still forms the basis for many membrane models, some of which of importance are shown in Figure 1. Figure 1A is a modification of Robertson's (1964) model of a three-layer structure with two monolayers of lipid with hydrocarbon chains. The chains are side by side and in end to end contact, and hydrophilic ends are oriented outwards and lined by the protein layers on either side. According to this model, the barrier to flow of material is the

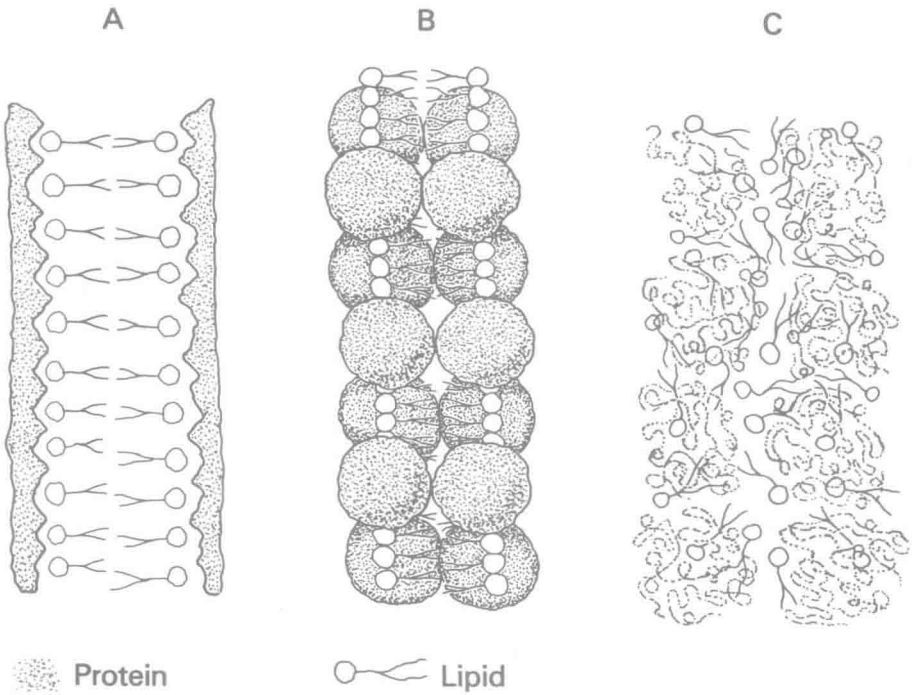

Figure 1. Models for the biological membranes. (A) Modification of the model due to Robertson (1964); (B) model suggested by Vanderkooi and Green (1970); (C) model according to the description of Stoeckenius and Engelman (1969). See text for details.

hydrophobic core, and the polar parts of the lipid and the proteins contain sites and/or enzymes involved in various membrane phenomena. The sites and proteins on either side of the central layer could be asymmetric. Figure 1B is a model suggested by Vanderkooi and Green (1970). The membrane consists essentially of two layers of globular protein molecules in contact with one another. The interspace between protein molecules is considered to be filled with phospholipids, with their polar groups remaining on the membrane surface and the fatty acid residues residing in the interspaces. The original Danielli–Davson model considered the core of the membrane to be highly ordered, but other data (Chapman *et al.*, 1968; Chapman and Wallach, 1968; Steim, 1968) indicate that the fatty acid residues exist in a state of disorder, giving the core layer properties typical of liquid hydrocarbons. The other situation, according to Stoeckenius and Engelman (1969), is indicated in Figure 1C; here the disordered hydrocarbon core is bounded by hydrophilic groups which are not closely packed. The hydrophilic surface of the lipid layer is only partially covered with proteins which are

not in extended β form but exist partly in α-helix conformation. The proteins also penetrate the lipid core of the membrane, whose integrity is governed by hydrophobic interactions between lipid and protein.

Despite the conception of the above interesting models, attempts at practical construction or preparation of these structures have failed, although bimolecular structures from lipids, both planar (black lipid membranes; Mueller *et al.*, 1962*a*,*b*; 1963) and spherical (Pagano and Thompson, 1967; Jung, 1971), have been formed. Incorporation of protein into these structures to form independently stable lipid–protein membranes has failed (Hanai *et al.*, 1965), although a supported protein–lipid–protein bilayer membrane has been formed (Tsofina *et al.*, 1966).

II. WATER AND IONS

As already mentioned, water forms nearly 80% of the total cell by weight, and so it should play a major role in regulating both the structure and function of the cell. Compared to other liquids, liquid water has relatively high boiling and melting points, suggesting the existence of strong intermolecular forces and a quasicrystalline structure. Spectroscopic studies of water in the gaseous state have shown that the molecule is nonlinear with H—O—H bond angle equal to 105°, and the internuclear distance between O and H atoms is 0.97 Å.

In gaseous oxygen atoms, there are six electrons in the second shell, two $2s$ electrons and four $2p$ electrons; the distinction between them becomes blurred as they enter into bond formation with hydrogen atoms. The two $1s$ electrons from the two atoms of hydrogen and the six electrons of the oxygen atom interact to form four sp^3 hybrid orbitals which are arranged in space directed towards the corners of a tetrahedron. Of the four electron orbitals, two remain as free orbitals for the lone pairs of electrons and the other two are used for the O—H bond. Due to repulsion of electron pairs, the H—O—H angle is not equal to the tetrahedral angle (109° 28′) but a little less (105°).

The free orbitals accommodating the two electron pairs confer on the water molecule two interesting properties: (1) It acts as an electric dipole because the centers of gravity of negative charges and of positive charges do not coincide. The dipole moment is 1.87×10^{-18} esu in the gas phase and becomes larger due to association with other water molecules. (2) It can associate with other water molecules since the two lone pairs of electrons can take part in electrostatic bonding and form hydrogen bonds with two other hydrogen atoms. Thus a cluster of water molecules can be formed. (See Figure 2 for a two dimensional representation of this association of

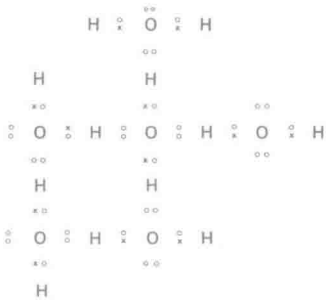

Figure 2. Association of water molecules. (×) Electron from the hydrogen atom and (○) electron from oxygen atom.

water molecules.) This is the situation that exists in a crystal of ice in which oxygen atoms form layers, each of which is a network of open but puckered hexagonal rings. Each oxygen atom is at the center of a tetrahedron with four other oxygen atoms at its corners. Between any two oxygen atoms is a hydrogen atom providing hydrogen bonding. The hydrogen atom at any instant is not located exactly half way between two oxygens. This network of associated water molecules has interstitial regions the dimensions of which are larger than that of a free unassociated water molecule. Consequently free water molecules can penetrate these interstitial spaces without disrupting the network structure. X-ray work has shown that the mean O—O distances for ice and liquid water are 2.76 and 2.92 Å, respectively. The number of oxygen nearest neighbors is 4 for ice and 4.4–4.6 for liquid water. These data thus indicate that liquid water still retains the lattice structure of ice, although it is slightly expanded. Free water molecules and network water molecules are in a state of dynamic equilibrium so that a water molecule breaking away from the network structure can enter the interstitial region and rotate freely. Similarly free water at any given moment can become part of the network at the next moment. The next point to consider is what happens to this picture of liquid water when ions enter it.

The symmetrical electrical field of the ion as it enters water will remove some of the water molecules from the water lattice and orient them in such a way that appropriate charged ends are facing one another. In fact a definite number of water molecules get trapped and orient toward the ion. Kinetically, they become part of the ion itself (primary ion hydration). Far away from the ion the normal structure of water (bulk water) remains undisturbed since the ionic field is attenuated. In the region between these two extremes, some water molecules are neither close enough to experience the full field strength of the ion nor distant enough to be part of the bulk water. Depending on their distance from the ion, they break away from the network and orient to varying degrees. So the water structure partially breaks down in this intermediate region (secondary ion hydration).

As the magnitude of ion hydration is determined by the ion field strength, large organic ions are considered to be without significant hydration.

III. MEMBRANE INTERFACES

The essence of all membrane models is to allow the polar groups of the phospholipid bilayer and those of the membrane protein to project into the aqueous phases surrounding them (membrane–solution interfaces). At physiological pH the polar groups are considered to be mostly negatively charged. Some water molecules will be associated with them. To maintain electroneutrality, cations (counterions) which are probably hydrated will exist close to the negative groups and form an electrical double layer which is characteristic of all phase boundaries. A crude extrapolation to the membrane–solution interface of the concepts that have evolved over the years by electrochemists for the double layer existing at a metal electrode–solution interface (Conway, 1965; Bockris and Reddy, 1970) is illustrated in Figure 3. In the compact part of the double layer there will be few coions (ions of the same charge as that on the membrane) and the concentration of counterions will be high compared to the concentration in the bulk solution. As one enters the diffuse part of the double layer, counter- and coions coexist.

Two broad aspects of electrical phenomena at interfaces deserve attention. The first refers to the consequences of having electrical charges at an interface in an electrolyte solution (see Figure 3), and the second concerns the nature of electrical potentials that occur at phase boundaries. Gouy-Chapman (see Grahame, 1947; Conway, 1965; Delahay, 1965) treated this problem and derived a relationship between the potential across the double layer, surface charge and concentration of ions in solution. This theory has been applied in recent years to membranes to explain shifts in conductance–voltage relations following changes in the concentration of divalent ions and, in so doing, has been used to derive values for the density of charges present on membranes (Gilbert and Ehrenstein, 1969; Gilbert, 1971; Mozhayeva and Naumov, 1970, 1972*a–c;* McLaughlin *et al.*, 1971; Muller and Finkelstein, 1972*a,b;* Brismar, 1973, D'Arrigo, 1973, Vogel, 1973; Begenisich, 1975; Hille *et al.*, 1975; Schauf, 1975). A brief account of the relations and their applications are given below.

If ψ is the potential at a distance x from the membrane surface, the local concentration n_i (molecules/cm^3) of ion i of valence z_i will be

$$n_i = n_i^0 \exp\left(-\frac{z_i e \psi}{KT}\right) \tag{1}$$

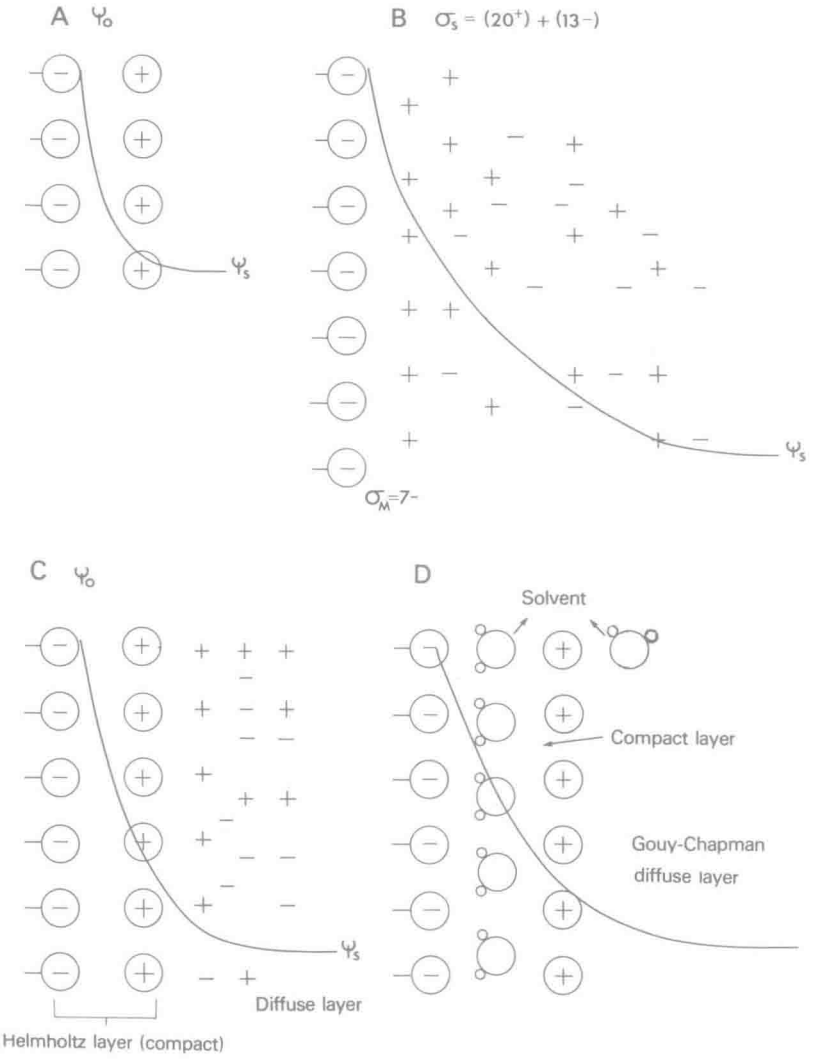

Figure 3. Structure of electrical double layer. (A) Helmholtz model. (B) Gouy model. Ion considered as a point charge. Surface charge per unit area indicated by cations (+) and anions (−). (C) Stern model. Finite size of the ion considered. (D) General representation of double layer with adsorption of water dipoles.

where n_i^0 refers to the bulk average stoichiometric concentration; e, the electronic charge; K, Boltzmann's constant; and T, the absolute temperature. The net charge density ρ_x at point x is given by

$$\rho_x = \sum_i z_i e n_i^0 \exp\left(-\frac{z_i e \psi}{KT}\right) \tag{2}$$

if ion distribution is governed by only electrostatic interactions. Poisson's equation relates ρ_x in one dimension to ψ by the equation

$$\frac{d}{dx}\left(\epsilon_x \frac{d\psi}{dx}\right) = -4\pi\rho_x \tag{3}$$

where ϵ_x is the dielectric constant.

Equation (3) takes into account any variation of ϵ_x in the double layer as a function of x (see Conway *et al.*, 1951; Grahame, 1950). Substitution of equation (2) in equation (3) gives

$$\frac{d}{dx}\left(\epsilon_x \frac{d\psi}{dx}\right) = -4\pi e \sum_i n_i^0 z_i \exp\left(-\frac{z_i e \psi}{KT}\right) \tag{4}$$

Using $\epsilon_x = \bar{\epsilon}$, a mean value of ϵ in the double layer (the value of ϵ_x is not very sensitive to variation of x except in the close vicinity of the membrane surface) and the identity

$$2\frac{d^2\psi}{dx^2} = \frac{d}{d\psi}\left(\frac{d\psi}{dx}\right)^2 \tag{5}$$

in equation (4) gives on integration

$$\frac{d\psi}{dx} = \left\{\frac{8\pi KT}{\bar{\epsilon}} \sum_i n_i^0 \left[\exp\left(-\frac{z_i e \psi}{KT}\right) - 1\right]\right\}^{1/2} \tag{6}$$

for the boundary conditions

$$\psi = 0, \qquad \frac{d\psi}{dx} = 0 \qquad \text{at } x = \infty$$

The surface charge density σ on the membrane is related to ρ_x by

$$\sigma = -\int_0^\infty \rho_x dx \tag{7}$$

If a is the distance of closest approach of the ions to the membrane, then integration of equation (3) and substitution into equation (7) gives the relation

$$\sigma = \frac{\epsilon_a}{4\pi}\left(\frac{d\psi}{dx}\right)_{x=a} \tag{8}$$

for the electric field at a, where ϵ_a is the dielectric constant in the region $x \leqslant a$. Equating ϵ_a to $\bar{\epsilon}$, although this assumption is unsatisfactory at the membrane surface, and substituting equation (8) in equation (6) gives, on rearrangement

$$\sigma = \left\{ \frac{KT\bar{\epsilon}}{2\pi} \sum_i n_i^0 \left[\exp\left(-\frac{z_i e \psi}{KT} \right) - 1 \right] \right\}^{1/2} \tag{9}$$

In most work, $\bar{\epsilon}$ is generally equated to the dielectric constant of bulk water, although this is considered very unsatisfactory (Conway, 1965; Bockris and Reddy, 1970). Expressing n_i^0 as $C_i N/1000$ (where N is the Avogadro number and C_i the bulk solution concentration, moles per liter), σ as the surface charge density in electronic charges per square angstrom, and $\psi = \psi_0$ at $x = a$, equation (9) at 22°C becomes

$$\sigma = \frac{1}{272} \left\{ \sum_i C_i \left[\exp\left(-\frac{z_i e \psi_0}{KT} \right) - 1 \right] \right\}^{1/2} \tag{10}$$

This equation becomes

$$\sigma = \frac{\sqrt{C}}{136} \sinh \frac{zF\psi_0}{2RT} \tag{11}$$

for ions of valence z, and

$$\sigma = \frac{1}{272} \left[C^+ \left(e^{F\psi_0/RT} + e^{-F\psi_0/RT} - 2 \right) + C^{2+} \left(2e^{F\psi_0/RT} + e^{-2F\psi_0/RT} - 3 \right) \right]^{1/2} \tag{12}$$

for solutions containing (1 : 1) and (2 : 1) electrolytes.

In the foregoing considerations, the binding of divalent ions to negative sites on the membrane was ignored. Gilbert (1971), Gilbert and Ehrenstein (1969, 1970), and McLaughlin *et al.* (1971) have taken this into account by considering the relation

$$C^{2+} + S^{2-} = CS; \qquad K_a = \frac{[CS]}{[C]\,[S]} \tag{13}$$

where C^{2+} is the concentration of divalent cation at the membrane containing sites of concentration S^{2-} and K_a is the association constant (liters per mole). The relation between the maximum charge density on the membrane (σ_{max}) in absence of divalent ions to that (σ) in presence of the ion is given by

$$\sigma = \frac{\sigma_{max}}{1 + K_a C_{bulk}^{2+} \exp\left(-2F\psi_0/RT\right)} \tag{14}$$

Equations (12) and (14) have been used to evaluate both σ_{max} and K_a. This consisted of making measurements of shifts in the relation between conductance (g_{Na} and/or g_K) and voltage (i.e., measurement of $\Delta V_{1/2}$ where $V_{1/2}$ is the potential at which conductance reached half the saturation value) following changes in calcium concentration from a standard solution (100 mM Ca-ASW) to a test solution (e.g., 10 mM Ca-ASW). This change, $\Delta V_{1/2}$, is used as a measure of the change in surface potential. Equations (12) and (14) are solved for two calcium concentrations with an iterative procedure by using different values of σ_{max} and of K_a until the best fit to the observed change (i.e., $\Delta V_{1/2}$) is obtained.

Some of the values for σ and K_a derived by a number of investigators for several membrane systems are given in Table 1.

When the potential is large ($-\psi_0 \gg RT/F$), equation (10) becomes

$$e^{-F\psi_0/RT} = \frac{272^2\sigma^2}{C^+} \quad \text{for monovalent ions} \tag{15}$$

$$= \frac{272\sigma}{\sqrt{C^{2+}}} \quad \text{for divalent ions} \tag{16}$$

$$= \frac{272^{2/3}\sigma^{2/3}}{\sqrt[3]{C^{3+}}} \quad \text{for trivalent ions} \tag{17}$$

This indicates that mono- and divalent ions will have the same effect on ψ_0 when

$$C^{2+} = \frac{(C^+)^2}{272^2\sigma^2} \tag{18}$$

Similarly di- and trivalent ions will have the same effect on ψ_0 when

$$(C^{3+})^2 = \frac{(C^{2+})^3}{(272\sigma)^2} \tag{19}$$

D'Arrigo (1973), by measuring the threshold for spike initiation in crayfish axons as a function of divalent (Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}) and trivalent cations (La^{3+}, Y^{3+}, Eu^{3+}), found that different cations of the same valence had the same effect on threshold membrane potential which, however, remained unchanged when a low concentration of trivalent cation (225 μM) or a high concentration of divalent cation (13.5 mM) was used. Consequently, use of these values of concentration in equation (19) gave a value of 1 e/43 Å^2 for σ, the charge density on the membrane of crayfish nerve.