phenomena will be obtained in further work with pulsed NMR studies which enable measurements of the relaxation times.

We thank Dr. H. J. C. Berendson for a copy of his manuscript (6) before publication.

This research was supported in part by a grant from the Washington State University Research Committee and in part by the United States Public Health Service grant CA 14496-01.

Received for publication 10 December 1972 and in revised form 5 June 1973.

REFERENCES

1. Magnuson, J. A., and N. S. Magnuson. 1972. *J. Am. Chem. Soc.* **94**:5461.
2. Ling, G. N., and F. W. Cope. 1969. *Science (Wash. D. C.).* **163**:1335.
3. Shporer, M., and M. M. Civan. 1972. *Biophys. J.* **12**:114.
4. Edzes, H. T., A. Rupprecht, and H. J. C. Berendson. 1972. *Biochem. Biophys. Res. Commun.* **46**:790.
5. Chen, D. M., and L. W. Reeves. 1972. *J. Am. Chem. Soc.* **94**:4385.
6. Berendson, H. J. C., and H. T. Edzes. 1972. *Ann. N. Y. Acad. Sci.* **204**:459.
7. Civan, M. M., and M. Shporer. 1972. *Biophys. J.* **12**:404.
8. Magnuson, J. A., D. S. Shelton, and N. S. Magnuson. 1970. *Biochem. Biophys. Res. Commun.* **39**:279.
9. Magnuson, J. A., and N. S. Magnuson. 1973. *Ann. N. Y. Acad. Sci.* **204**:297.
10. Osborn, M. J. 1972. *In* Structure and Function of Biological Membranes. L. I. Rothfield, editor. Academic Press Inc., New York. 343.
11. Bull, T. E. 1972. *J. Magn. Resonance.* **8**:344.

Nancy S. Magnuson
James A. Magnuson
Graduate Program in Biochemistry
Department of Chemistry
Washington State University
Pullman, Washington 99163

The Double Fixed Charge Membrane Model: an Hypothesis Concerning the Structure and Morphogenesis of Cell Membranes

Dear Sir:

The electrical properties of a membrane containing two fixed charge regions, of opposite sign, in juxtaposition, show many similarities to those of cell membranes (e.g., Coster, 1965, 1969, 1973 *a*, *b*). In the theoretical analysis of this membrane model previously given (e.g., Mauro, 1962; Coster, 1965, 1973 *a*, *b*), neither the question of a possible physical realization of the model system, nor the relationship between the model and the structures postulated for the cell membrane, was considered.

Based on the hypothesis of the presence of a double fixed charge system in cell membranes, we now wish to put forward an hypothesis of the molecular organization, morphogenesis, and appearance, under electron microscopy, of the cell membrane.

Proteins, either as a separate phase or in various lipoprotein and glycoprotein complexes are a major constituent of all cell membranes. For the present discussion they are, as suggested by much recent work (e.g., see Fox, 1972), assumed to be distributed throughout the bulk of the membrane. Fixed charges in the cell membrane could arise on the membrane proteins from the ionization of NH_2 or COOH groups not involved in the peptide links (i.e., on basic and acidic amino acids).

For the double fixed charge membrane (FCM) model we require that under normal physiological conditions one-half of the membrane contains a predominance of positive sites and the other a predominance of negative sites. Such a distribution of ionized sites, in principle, could, for example, reflect an intrinsic asymmetry of ionizable sites on the proteins, perhaps as a consequence of the generation of the membrane in the presence of the very large electrochemical gradients which exist between the cytoplasm and the external medium. Alternatively, or additionally, it could also reflect differences in pH between the external medium and the cell interior.

The junction of a fixed positive charge lattice with the fixed negative charge lattice gives rise to a transition region, the depletion layer, which is almost completely depleted of mobile ions and in which the space charge is consequently very high (Mauro, 1962; Coster, 1965).

The precise form of the profiles of the concentrations of mobile ions permeating such a membrane and the electrostatic potential depends on the distribution of fixed ionized sites, the dielectric constant of the membrane, the concentration of ions in the external solutions, and the cellular potential developed across the membrane (e.g., see Coster et al., 1969; Coster, 1973 *b*). The latter is in turn dependent on the solution concentration as well as the diffusion constants for the various ions in the membrane.

The electric field strength in the depletion layer, which under physiological conditions would be ~30 Å in thickness, is very high. For a fixed charge concentration of ~0.1 M with a dielectric constant of 10, under physiological conditions, the field in the depletion layer, both for a graded and an abrupt fixed charge density profile would be of the order of 10^8 V/m, even with zero potential across the membrane (e.g., see Coster et al., 1969).

Pursuing the assumption that the cell membrane does indeed contain a double fixed charge system it is suggested that:

(*a*) The very high electric field strength in the depletion layer of the double FCM would lead to an alignment, transverse to the membrane, of many long chained polypeptide, lipoprotein, glycoprotein, and phopholipid molecules. This is at once apparent when one considers that the energy difference for the aligned and nonaligned orientations of a molecule with an electric dipole moment as small as 4×10^{-29} cm (i.e., an electronic charge separation of ~2.5 Å) in an electric field of 10^8 V/m is larger than the thermal energy kT (at 18°C).

(*b*) An alignment of the molecules in the strong field would also result in a much more closely packed arrangement of the molecules.

(*c*) In the regions outside the depletion layer the electric field is relatively weak and the proteins would likely be randomly coiled, α-helical, or globular in nature.

(*d*) At very high field strengths the dielectric constant, which is a reflection of the electric polarizability of the molecular constituents, decreases due to saturation effects (e.g., see Booth, 1955; Buckingham, 1956). It would thus be likely that the dielectric constant of the aligned molecules in the depletion layer could be significantly lower than in the adjacent low field regions.

(*e*) The electric field in the depletion layer of a double FCM is not constant and has a maximum value in the center of this region. From energy considerations, molecules with a residual dipole moment in such a nonuniform field would tend to accumulate in the regions of maximum field strength. This effect could, of course, be largely offset by considerations of hydrophilic and hydrophobic bonding involved in the final distribution of material. The electrostatic effect would, furthermore, more likely be significant for molecules of relatively small chain length, such as lecithin or cholesterol, rather than the high molecular weight polypeptides.

Unlike the scheme proposed in the Danielli-Davson-Robertson model (Danielli and Davson, 1935; Robertson, 1959), the gross morphology would thus on this model reflect the antecedent electrochemical profiles and would not result from a dimensionally fixed and defined bimolecular lipid leaflet.

(*f*) Based on the assumption of a bipolar distribution of ionized sites it is proposed that the central high field region containing transversely aligned molecular chains, sandwiched between the two weak field regions containing more randomly coiled protein molecules, corresponds to the trilayer structure of cell membranes seen under electron microscopy.

A quantitative comparison of the trilaminar cell membrane structure and the three regions which are generated in the double FCM can be made by calculation of the width of the depletion layer (for details see Coster [1965] taking into account the effect of partition coefficients, Coster [1973 *b*]).

A direct comparison of the dimensions of the theoretical profiles and the resultant molecular organization and the membrane structure revealed in the electron micrographs of OsO_4-fixed cells of *Chara corallina* is shown in Fig. 1. The values of the fixed charge parameters used in calculating the profiles were: fixed charge concentrations 0.1 M, dielectric constant 10, and a product of 1 mM for the external ion concentrations and the ion partition coefficients. These values are not only physiologically reasonable but were also required to fit the theoretical DC and AC characteristics to typical experimental results obtained for these cells.

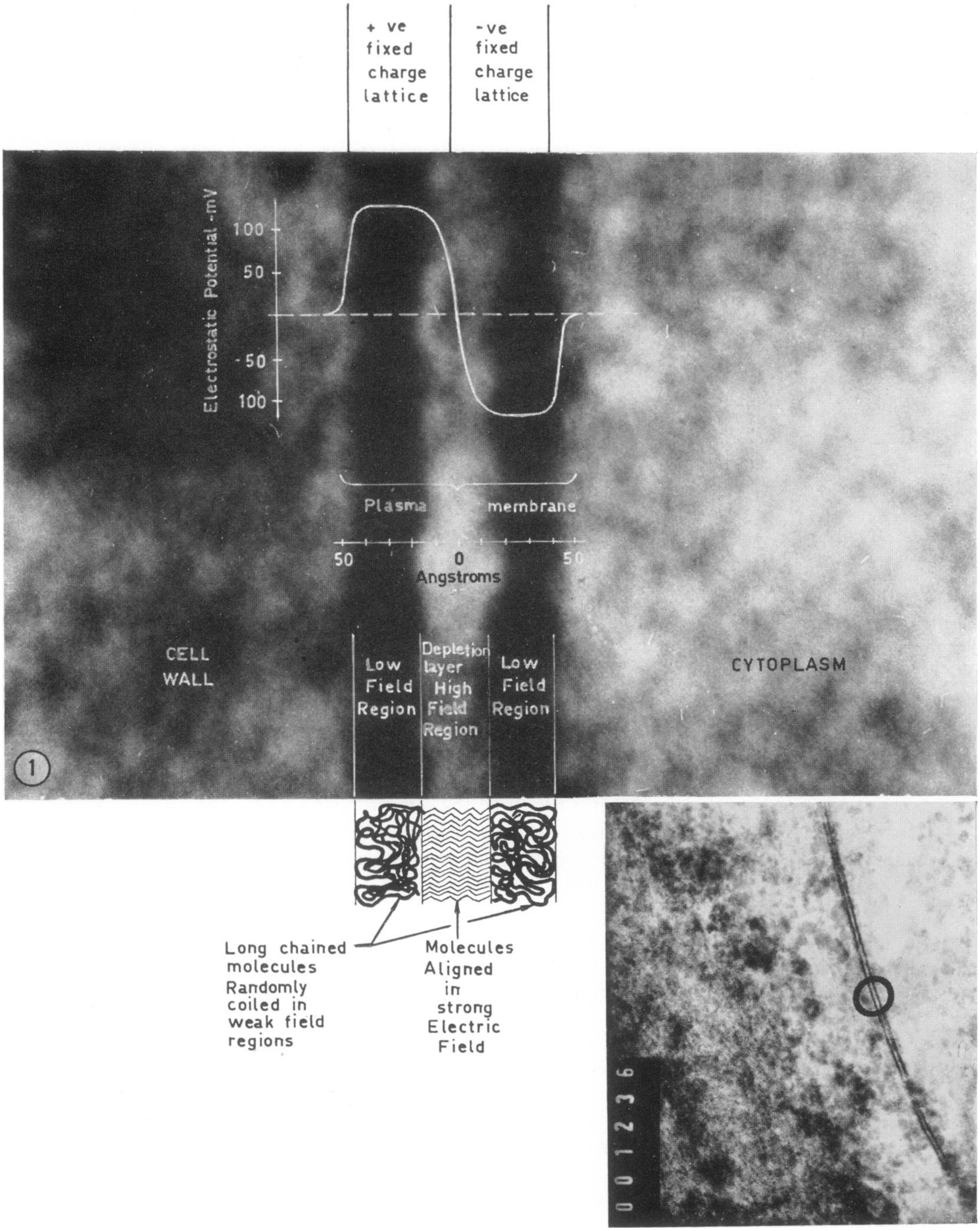
It is evident from the figure that the theoretical widths of the central high field (depletion) layer and the adjacent low field regions, indicated on the electron micrograph, closely correspond the central electron-lucent and adjacent electron-dense regions, respectively, in the OsO_4-fixed membrane.

On the hypothesis proposed the dimensions of the trilayer structures seen in cellular membranes are easily accounted for by variations in the fixed charge parameters such as, for instance, the fixed charge concentration (through variation in the amino acid composition of membrane proteins or external pH), or the dielectric constant (e.g., through variations in the lipid content).

The pH which is known to have profound effects on the electrical and permeation properties of cell membranes would thus also perhaps be expected to have an affect on the membrane structure. Indeed a dependence on pH of the relative widths of the three layers in the plasma membrane structure has already been observed in electron microscope studies of the membranes of cells of *C. corallina* cultured and fixed at different pH (Coster and Kaplin, in preparation).

The observations of Fleisher and Stoeckenius (1967) that membranes from which 90% of the lipids had been extracted still displayed the trilayer structures seen in the original membranes would, further, not be unexpected with the double FCM model. This would not deny the importance of lipids in the membrane, for instance through their effect on the dielectric constant. The latter quantity would have a twofold effect. Firstly the width of the depletion layer in the double FCM is a function of the dielectric constant for a given potential difference appearing across the depletion layer. This would thus also reflect in the molecular structure and morphology of the membrane. Secondly a decrease in the dielectric constant would increase the solution-membrane ion partition coefficients and hence increase the "Donnan" potentials at each solution-membrane interface (Coster, 1973 *b*). This would thus enhance the bipolar nature of the membrane profiles (and hence also its stability).

Finally, it is worth noting that given the formation of an initial, perhaps amorphous, membranous layer in which either an intrinsic or induced asymmetrical profile of concentration of ionized sites is present, the morphogenesis of the molecular organization and trilayer structure would proceed spontaneously.



①

+ ve fixed charge lattice
- ve fixed charge lattice
Electrostatic Potential -mV
100
50
-50
100
Plasma membrane
50
0
50
Angstroms
CELL WALL
Low Field Region
Depletion layer High Field Region
Low Field Region
CYTOPLASM
Long chained molecules Randomly coiled in weak field regions
Molecules Aligned in strong Electric Field
0 0 1 2 3 6

The author wishes to thank the Australian Research Grants Committee for support for the conduct of this research.

Received for publication 8 December 1972 and in revised form 11 May 1973.

REFERENCES

BOOTH, F. 1955. *J. Chem. Phys.* **23**:453.
BUCKINGHAM, A. D. 1956. *J. Chem Phys.* **25**:428.
COSTER, H. G. L. 1965. *Biophys. J.* **5**:669.
COSTER, H. G. L. 1969. *Aust. J. Biol. Sci.* **22**:365.
COSTER, H. G. L. 1973 *a. Biophys. J.* **13**:118.
COSTER, H. G. L. 1973 *b. Biophys. J.* **13**:133.
COSTER, H. G. L., E. P. GEORGE, and R. SIMONS. 1969. *Biophys. J.* **9**:666.
DANIELLI, J. F., and H. J. DAVSON. 1935. *J. Cell Physiol.* **5**:495.
FLEISHER, S. B., and W. J. STOECKENIUS. 1967. *J. Cell Biol.* **32**:193.
FOX, C. F. 1972. *Sci. Am.* **226**:30.
MAURO, A. 1962. *Biophys. J.* **2**:179.
ROBERTSON, J. D. 1959. *Biochem. Soc. Symp.* **16**:3.

H. G. L. COSTER
School of Physics
University of New South Wales
Sydney, Australia 2033

FIGURE 1 A comparison of the theoretical profiles and suggested resultant molecular organization in the double FCM model, with the trilaminar structure of the cell membrane seen under electron microscopy. The theoretical profile shown is for an abrupt junction double FCM with the parameters listed in the text, at zero membrane potential (zero potential for comparison with an OsO_4-fixed cell). The values chosen are both physiologically reasonable and were required to fit the theoretical AC and DC electrical characteristics of the double FCM to typical experimental results for the membranes of cells of *C. corallina* (Coster, 1965, 1969, 1973 *a*, *b*). The electron micrographs show the plasma membrane of these cells fixed in OsO_4. The large electron micrograph on which the theoretical profiles for the double FCM have been plotted is a photographically enlarged segment of the central portion of the encircled area in the electron micrograph shown in the *inset*. The scale shown in the larger print was determined by calibration of the electron microscope using a diffraction grating. The photographic enlargement factor was determined optically. The depletion layer in the theoretical profiles, indicated on the electron micrograph, where the electric field strength is very high and the molecules would tend to align transversely to the membrane, is here identified with the electron-lucent central region in the OsO_4-fixed membrane. The outer low field regions in the double FCM, where the molecules would more likely be randomly coiled, are identified with the electron-dense regions in the OsO_4-fixed membrane. (Top, ×98,000; inset, ×3,400,000.)