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Estimation of Surface Charges in Some Biological Membranes

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Summary. The resting membrane potential data existing in the literature for the giant axon of the squid, frog muscle and barnacle muscle have been analyzed from the standpoint of the theory of membrane potential due to Kobatake and co-workers. The average values derived for the effective charge density $\bar{\phi}\overline{X}$ (where $\bar{\phi}$ is a constant, $0<\bar{\phi}<1$, and represents the fraction of counterions that are free, and $\overline{X}$ is the stoichiometric charge density in the membrane) present on the different biomembranes existing in their normal ionic environment are 0.3, 0.325 and 0.17 M for the squid axon, frog and barnacle muscles, respectively. On the assumption that the values of $\bar{\phi}$ are 0.4 and 0.2 for nerve and muscle membranes, respectively, values of 0.75, 1.62 and 0.85 M have been derived for the stoichiometric charge density ($\overline{X}$) present in the respective biological membranes. These correspond to 1 negative charge per 222, 103 and 195 Å^2 of the membrane area of the squid axon, frog and barnacle muscles, respectively.

In a recent paper, Lakshminarayanaiah (1975*a*) applied the theory of membrane potential developed by Kobatake and colleagues (*see* Kobatake & Kamo, 1973 for a review) to derive values for the effective fixed-charge density of artificial ion exchange membranes. It was shown that this theory (K-theory) gave more reliable values for the effective fixed-charge density of membranes than an older theory (TMS) due to Teorell (1953) and Meyer and Sievers (1936) whose theoretical and technical limitations have been discussed elsewhere (Lakshminarayanaiah, 1974). However, both the theories are of a general nature and can be applied to thick (0.2–0.5 mm) as well as thin ($\sim$50–100 Å) membranes provided the membranes have ionogenic groups to generate and to sustain electrical potentials across them when they separate electrolyte solutions of different concentrations.

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The ability of membranes to prefer cations and/or anions (i.e. permselectivity) depends both on the concentration of ionogenic groups in the membrane and the concentration of the surrounding electrolyte solutions. Artificial membranes that prefer cations or anions because they carry anionic or cationic groups show little preference to any one particular ion unless they have been doped with special compounds. Therefore, under normal conditions, whatever the charge density, artificial membranes when surrounded by an electrolyte environment in which the biological membranes exist (high concentration of potassium on one side, i.e. intracellular, and high concentration of sodium on the other side, i.e. extracellular) show no ability to sustain either the equilibrium potential due to potassium ions, that is

$$E_{\max} = \frac{RT}{F} \ln \frac{(\mathrm{K})_o}{(\mathrm{K})_i} \tag{1}$$

or the potential due to sodium ions. The net potential (i.e. the bi-ionic potential) observed across the membrane will be determined simply by the concentrations, mobilities of both cations (K, Na) and anions and their activity coefficients in the membrane phase (Lakshminarayanaiah, 1969*a*). On the contrary, biological membranes of nerve (e.g. squid axon) and muscle (e.g. frog and barnacle) in their resting state maintain a potential across them which is determined mostly by the concentrations of intra- and extracellular potassium. This is possible only if the Na outside is prevented from entering the biological membrane phase. Some special property of the membrane and/or of its ionogenic groups probably is responsible for this.

A number of investigators have measured the resting potential across the squid axon membrane as a function of the concentration of potassium by changing it both externally and internally. Similarly, the resting membrane potential across the membrane of the frog (sartorius and semitendinosus) has been followed as a function of the changes in the external potassium concentration. The results of these measurements of membrane potential have been used in this paper to derive values for the effective fixed-charge density in membranes of the squid nerve and frog muscles by applying the K-theory. In a recent Letter-to-the-Editor, Lakshminarayanaiah (1975*b*) described the results following from the application of the K-theory to the membrane potential data derived for the barnacle muscle membrane (Lakshminarayanaiah, 1974).

Results

Giant axons of the squid have been used by a number of investigators to study the resting membrane properties as well as its excitability characteristics. In Tables 1–5 are given some of the resting membrane potential data derived for the squid axon membrane. In Tables 6 and 7 are given similar data derived for the frog muscle membranes.

Table 1. Membrane potential across intact squid axon membrane (Hodgkin & Keynes, 1955)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$[a]	E_m[b] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)][c]	ξ [Eq. (5)]	$\overline{\phi X}$ [Eq. (6)] (moles/kg H_2O)
3.5	378	−63	−118.3	0.766	0.547	0.765	0.249
10.4	378	−64	− 90.8	0.853	0.715	0.489	0.397
21.6	378	−53	− 72.3	0.867	0.742	0.451	0.443
60.6	378	−43	− 46.2	0.965	0.933	0.193	1.134
217	378	−17	− 14.0	1.106	1.203	–	–
493	378	+ 1	6.7	0.575	0.169	2.925	0.149

[a] Value of $(K)_i$ is the average concentration determined at different periods after dissection (Steinbach & Spiegelman, 1943).
[b] Values of E_m taken from Table 2 (Hodgkin & Keynes, 1955).
[c] The value used for t_+ in the evaluation of P_s is 0.49.

Table 2. Membrane potential across internally perfused squid axon membrane (Baker, Hodgkin & Shaw, 1962)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$	E_m[a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)][b]	ξ [Eq. (5)]	$\overline{\phi X}$ [Eq. (6)] (moles/kg H_2O)
10.1	29.4	−11.8	− 26.99	0.719	0.453	0.983	0.020
10.1	88.0	−29.8	− 54.7	0.773	0.559	0.742	0.066
10.1	175	−44.5	− 72.0	0.809	0.630	0.617	0.150
10.1	338	−53.0	− 88.7	0.799	0.611	0.649	0.268
10.1	622	−57.5	−104.1	0.776	0.566	0.728	0.434
111	175	− 9.0	− 11.5	0.891	0.790	0.388	0.369
111	338	−26.5	− 28.1	0.971	0.944	0.174	1.290
111	622	−36.5	− 43.5	0.919	0.844	0.318	1.155
546	88	+13.5	+ 46.1	0.646	0.311	1.528	0.207
546	622	− 3.75	− 4.9	0.885	0.779	0.403	1.499

[a] Values of E_m read off from Fig. 2 (Baker *et al.*, 1962).
[b] The value used for t_+ in the evaluation of P_s is 0.49.

Table 3. Membrane potential across internally perfused squid axon membrane (Narahashi, 1963)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$ (mmoles/kg H_2O)	E_m [a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)] [b]	ξ [Eq. (5)]	$\phi\overline{X}$ [Eq. (6)] (moles/kg H_2O)
10.1	75.4	−40.3	− 50.8	0.897	0.801	0.374	0.114
10.1	602 [c]	−64.3	−103.3	0.811	0.635	0.608	0.503
10.1	1180 [c]	−62.8	−120.3	0.761	0.537	0.786	0.757

[a] Values of E_m taken from Table 2 (Narahashi, 1963).
[b] The value used for t_+ in the evaluation of P_s is 0.49.
[c] Mixture of KCl and K_2SO_4 used.

Table 4. Membrane potential across internally perfused squid axon membrane (Baker, Hodgkin & Meves, 1964)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$ (mmoles/kg H_2O)	E_m [a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)] [b]	ξ [Eq. (5)]	$\phi\overline{X}$ [Eq. (6)] (moles/kg H_2O)
10.1	7.4	+ 5.0	7.86	0.818	0.648	0.587	0.015
10.1	29.4	−14	− 27.0	0.759	0.533	0.793	0.025
10.1	120	−34	− 62.5	0.772	0.558	0.744	0.087
10.1	338	−41	− 88.7	0.731	0.478	0.919	0.189
10.1	622	−49	−104.1	0.735	0.486	0.899	0.352
21.6	7.4	+11	27.1	0.703	0.423	1.071	0.014
21.6	29.4	− 7	− 7.8	0.950	0.903	0.238	0.107
21.6	120	−27	− 43.3	0.812	0.636	0.608	0.117
21.6	338	−40	− 69.5	0.788	0.589	0.686	0.262
21.6	622	−45	− 84.9	0.765	0.544	0.770	0.418

[a] Values of E_m read off from Fig. 3 (Baker *et al.*, 1964).
[b] The value used for t_+ in the evaluation of P_s is 0.49.

The concentrations of $(K)_o$ and $(K)_i$ given in the Tables have been converted to the molal scale (moles/kg water) using appropriate density data (Weast, 1972–73). In column 3 are given the values of E_m, the membrane potential measured by various investigators. The values given in columns 4 and 5 have been calculated by using, respectively, Eq. (1) and the equation (Teorell, 1936; Lakshminarayanaiah, 1969*b*)

$$\bar{t}_{+(app)} = \frac{E_m}{2E_{max}} + 0.5 \tag{2}$$

Table 5. Membrane potential across internally perfused squid axon membrane (Rojas, Atwater & Bezanilla, 1970)

$(KF)_o$ (mmoles/kg H_2O)	$(KF)_i$	E_m [a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)]	ξ [Eq. (5)]	$\phi\overline{X}$ [Eq. (6)] (moles/kg H_2O)
23.8	552	−41.2	−79.4	0.759	0.408	1.119	0.257
119	552	−26.0	−38.8	0.835	0.586	0.692	0.485
188	552	−12.1	−27.2	0.722	0.323	1.466	0.253
The value used for t_+ in the evaluation of P_s is 0.57.							
$(KCl)_o$	$(KF)_i$						
11.9	552	−40.1	−96.9	0.707	0.362	1.286	0.219
95.2	552	−19.9	−44.4	0.725	0.399	1.148	0.282
314	552	− 4.7	−14.3	0.665	0.275	1.746	0.248
The value used for t_+ in the evaluation of P_s is 0.53.							

[a] Values of E_m read off from Fig. 4 (Rojas *et al.*, 1970).

Table 6. Membrane potential across frog sartorius muscle membrane (Adrian, 1956)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$	E_m [a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)] [b]	ξ [Eq. (5)]	$\phi\overline{X}$ [Eq. (6)] (moles/kg H_2O)
0.5	139	−120	−142.1	0.922	0.850	0.309	0.226
1.0	139	−110	−124.6	0.941	0.887	0.260	0.269
2.5	139	− 92	−101.5	0.953	0.910	0.228	0.311
5.0	139	− 79	− 83.9	0.971	0.944	0.174	0.413
10.0	139	− 61	− 66.4	0.959	0.922	0.210	0.354
25.1	139	− 42	− 43.3	0.986	0.972	0.121	0.680
50.3	139	− 26	− 25.7	1.006	1.012	–	–

[a] Values of E_m read off from Fig. 5 (Adrian, 1956).
[b] The value used for t_+ in the evaluation of P_s is 0.49.

where $\bar{t}_{+(app)}$ is the apparent transport number of the K ion in the membrane phase. In the calculation of E_{max}, a value of 20 °C has been used for temperature T in all cases due to lack of information about the exact temperature at which the potential measurements were made. This will introduce some error into the values of $\phi\overline{X}$ (effective fixed-charge density). Some sample calculations made at 10 °C indicate that the value of $\phi\overline{X}$ will be higher by about 10% of that calculated at 20 °C. Most of the measurements seem to have been carried out between 15° and 20 °C.

Table 7. Membrane potential across frog semitendinosus muscle membrane (Hodgkin & Horowicz, 1959)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$	E_m[a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)]	ξ [Eq. (5)]	$\bar{\phi X}$ [Eq. (6)] (moles/kg H_2O)
2.5	139	− 93.5	−101.5	0.961	0.924	0.207	0.342
5.0	139	− 77.2	− 83.8	0.960	0.924	0.207	0.348
10.0	139	− 62.0	− 66.5	0.966	0.935	0.189	0.394
30.2	139	− 38.6	− 38.6	1.000	1.000	–	–
75.5	139	− 16.2	− 15.4	1.025	1.049	–	–
192	139	+ 2.0	+ 8.2	0.623	0.264	1.827	0.091
The concentration of KCl was varied keeping the product $(K)_o(Cl)_o$ constant. The value used for t_+ in the evaluation of P_s is 0.49.							
0.5	139	−113.5	−142.1	0.900	0.813	0.358	0.195
1.0	139	−104.8	−124.6	0.920	0.852	0.307	0.228
2.5	139	− 92.3	−101.5	0.955	0.916	0.219	0.323
5.0	139	− 79.8	− 83.8	0.976	0.955	0.155	0.466
10.0	139	− 66.5	− 66.5	1.000	1.000	–	–
20.1	139	− 48.0	− 48.8	0.991	0.984	0.090	0.882
40.2	139	− 30.0	− 31.3	0.979	0.961	0.145	0.619
80.5	139	− 15.0	− 13.8	1.044	1.080	–	–
$(K)_o$ was varied in the absence of chloride by using sulfate solution. The value used for t_+ in the evaluation of P_s is 0.48.							

[a] The values of E_m read off from Figs. 4 and 5 for the top and bottom entries, respectively (Hodgkin & Horowicz, 1959).

Values for the permselectivity P_s given in column 6 were calculated from the equation (Lakshminarayanaiah, 1975*a*)

$$P_s = \frac{\bar{t}_{+(app)} - t_+}{t_+ - \bar{t}_{+(app)}(2t_+ - 1)} \tag{3}$$

where t_+ is the transport number of the cation in the aqueous phase.

In the literature concerned with artificial membranes, permselectivity is usually expressed as (Winger, Bodamer & Kunin, 1953)

$$P_s = \frac{\bar{t}_i - t_i}{1 - t_i} \tag{4}$$

where $\bar{t}_i$ and t_i are the transport numbers of counterions i in the membrane and in the bulk solution, respectively. Eq. (4) defines permselectivity in an arbitrary manner and does not relate it in any way to the fixed-charge density of the membrane. On the other hand, Eq. (3) relates it to the fixed-

charge density of the membrane by the equation (Lakshminarayanaiah, 1975*a*)

$$\xi = (\sqrt{1 - P_s^2})/2P_s \tag{5}$$

where ξ is given by

$$\bar{\phi}\overline{X} = \frac{(K)_o + (K)_i}{2\xi}. \tag{6}$$

$\bar{\phi}$ is a constant ($0 < \bar{\phi} < 1$) representing the fraction of counterions that are not closely associated with the fixed negative groups of stoichiometric concentration $\overline{X}$ in the membrane.

When the cationic transference number is 0.5 in the bulk aqueous solution (e.g. KCl), Eq. (3) reduces to

$$P_s = \frac{\bar{t}_{+(app)}}{0.5} - 1. \tag{7}$$

$\bar{t}_{+(app)}$ can have values from 0.5 (cation transference number in the bulk KCl solution) to 1.0. It can not have a value less than 0.5 unless the membrane has become an anion exchanger in which case the membrane becomes more permeable to anions than to cations. It follows then, that the value of P_s is always finite ranging in value from 0 to 1.

In the calculation of the values for t_+, the limiting mobilities of K^+ ion and that of the concerned anion have been used. In columns 7 and 8 of Tables 1–9, are given the values of ξ and $\bar{\phi}\overline{X}$, respectively.

Table 8. Membrane potential across perfused barnacle muscle membrane (Lakshminarayanaiah & Rojas, 1973)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$	E_m [a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)] [b]	ξ [Eq. (5)]	$\bar{\phi}\overline{X}$ [Eq. (6)] (moles/kg H_2O)
10.1	117	−45.8	− 61.9	0.870	0.634	0.610	0.104
10.1	170	−54.4	− 71.3	0.881	0.664	0.563	0.160
10.1	230	−56.0	− 78.9	0.855	0.594	0.678	0.177
10.1	338	−60.2	− 88.7	0.840	0.554	0.751	0.232
10.1	440	−67.0	− 95.3	0.851	0.585	0.693	0.325
10.1	530	−67.1	−100.0	0.835	0.544	0.772	0.350
10.1	665	−71.1	−105.8	0.836	0.546	0.768	0.439

[a] Values of E_m taken from Table 3, column 2 (Lakshminarayanaiah & Rojas, 1973).
[b] A value of 0.6 for t_+ has been used in the evaluation of P_s.

Table 9. Membrane potential across perfused barnacle muscle membrane (Lakshminarayanaiah & Rojas, 1973)

$(K)_o$ (mmoles/kg H_2O)	$(K)_i$	E_m[a] (mV)	E_{max} [Eq. (1)] (mV)	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)][b]	ξ [Eq. (5)]	$\phi\overline{X}$ [Eq. (6)] (moles/kg H_2O)
0[c]	230	−60.5	−195.5	0.655	0.117	4.258	0.027
10.1	230	−56.0	− 78.9	0.855	0.594	0.678	0.177
25.2	230	−42.2	− 55.9	0.878	0.655	0.578	0.221
50.3	230	−32.3	− 38.4	0.921	0.771	0.413	0.339
100.7	230	−22.7	− 20.9	1.044	1.135	—	—
151.0	230	−15.1	− 10.6	1.210	1.705	—	—
201.3	230	− 8.1	− 3.4	1.703	4.252	—	—

[a] Values of E_m taken from the work of Lakshminarayanaiah and Rojas (1973) pertaining to Fig. 3.

[b] A value of 0.6 for t_+ has been used in the evaluation of P_s.

[c] A value of 0.1 has been used for $(K)_o$ in the calculation of E_{max}.

Discussion

Recently, it was shown by Lakshminarayanaiah (1975*a*) that in the case of cross-linked sulfonated phenol-formaldehyde and polymethacrylic acid, membranes whose density of charges were determined both analytically (values of $\overline{X}$) and potentiometrically (values of $\phi\overline{X}$), the values of both $\overline{X}$ and $\phi\overline{X}$ increased with increase in the concentration of the surrounding electrolyte solutions. At any given concentration, the value of $\phi\overline{X}$ was less than the value of $\overline{X}$. However, with increasing ambient electrolyte concentration, the value of $\phi\overline{X}$ showed a tendency to reach the quantitative value of $\overline{X}$. The values of $\phi\overline{X}$ given in Tables 1–7 for the biological systems (except the values given in Table 5) show the same behavior described above for the model ion exchange membranes although we do not know of any value of $\overline{X}$ with which to compare the values of $\phi\overline{X}$ in these biological systems.

In the case of model systems, the low value of $\phi\overline{X}$ in solutions of low ionic strength has been attributed to the close association of counterions with the ionogenic groups (Lakshminarayanaiah, 1975*a*). There will be few co-ions in the membrane phase and thus the double layer formed of negative groups of the membrane and the associated counterions will be compact and clear. With increase in the ionic strength, co-ions along with counterions to maintain electroneutrality, will invade the region of the double layer and thereby reduce the thickness of the double layer. This tendency will increase with further increase in electrolyte concentration

and thus eliminate the distinction between counterions and co-ions. In this case, the region near the membrane charges would not be any different from the region away from them. The positive and negative species in the system will behave like they would in an ordinary electrolyte solution of comparable ionic strength. In other words, the fraction of counterions that are not closely associated with the fixed negative groups in the membrane (i.e. the value of $\bar{\phi}$) tends to increase with increase in the external electrolyte concentration. Similar behavior is also shown by the parameter $\overline{X}$ (see last column of Table 1 of Lakshminarayanaiah, 1975*a*) in the case of ion exchange membranes used by Lakshminarayanaiah (1975*a*). In the case of other ion exchange membranes in which the membrane undergoes little dimensional change (i.e. no change in water content) following change in the external solution concentration, the value of $\overline{X}$ would remain constant. In either case, the value of $\bar{\phi}\overline{X}$ would increase with increase in the concentration of the surrounding electrolyte solution. Under these conditions, $\bar{\phi}$ will tend to reach its limit of unity and thereby will eliminate the influence of the ionogenic groups on the counterions in the membrane. This results in complete loss of membrane permselectivity (i.e. $P_s = 0$). Therefore it is suggested that the same explanation, namely the decrease in the extent of association of counterions with the charges in the membrane following an increase in the concentration of the surrounding electrolyte solution, may be offered to explain the increase in the value of $\bar{\phi}\overline{X}$ in the biological membranes.

The squid axon in its normal resting state has about 400 mM for $(K)_i$ and 10 mM for $(K)_o$ (Lakshminarayanaiah, 1969*c*). The value of $\bar{\phi}\overline{X}$ corresponding to this ambient concentration lies between 0.25 and 0.40 M. The average value is about 0.3 M (i.e. 0.397 from Table 1; 0.3 from Table 2; 0.35 from Table 3; 0.25 from Table 4 and 0.22 from Table 5). Similarly, the frog muscle in its normal ionic environment [$(K)_i = 139$ mM, $(K)_o = 2.5$ mM (Adrian, 1956)] has an average value of 0.325 M (i.e. 0.311 from Table 6; 0.342 and 0.323 from Table 7). Unfortunately the value of $\bar{\phi}$ is unknown for the biological systems considered in this paper. Without this value, the values derived for the effective fixed-charge density $\bar{\phi}\overline{X}$ assume little significance. In order to remove this lacuna, an assumption about the value of $\bar{\phi}$ can be made; and that is to assume that the extent of influence which the ionogenic groups in the biological membrane exert on the K^+ counterions is the same as that observed in the carboxylic acid ion exchange membranes. It is generally believed that the negatively charged groups in the biological membranes are the carboxylic groups (Mozhayeva & Naumov, 1970; 1972; Hille, 1973; Woodhull, 1973). Although

the physiological environment in which the carboxylic groups exist in the biological membrane is different from the environment in which the model ion exchange membrane systems are used, the ionic environment can be made similar to some extent in respect of its strength despite the absence of divalent Ca and Mg ions in solutions used in model systems. So, it is not unreasonable, in the absence of other valid alternatives, to make the above assumption regarding the value of $\bar{\phi}$. The data given by Lakshminarayanaiah (1975*a*) show that for the average ambient concentrations existing in squid ($\sim$200 mM) and frog muscle ($\sim$70 mM), the values of $\bar{\phi}$ are about 0.4 and 0.2, respectively. This leads to values of 0.75 and 1.62 M for $\bar{X}$ in the case of membranes of the squid nerve and frog muscle, respectively. If the assumption made earlier (Lakshminarayanaiah, 1974; 1975*b*) that the water associated with the polar groups of the membrane lipid bilayer and the enveloping proteins is 10 Å thick, is accepted, then 0.75 M corresponds to a distribution of one negative charge per 222 Å^2 membrane surface of the squid axon and 1.62 M corresponds to a distribution of one negative charge per 103 Å^2 membrane surface of the frog muscle.

In the case of the barnacle muscle fiber, none of the membrane potential data presented earlier (Lakshminarayanaiah, 1974) correspond to the normal ionic environment which is $(K)_i = 180$ mM and $(K)_o = 10$ mM. Further, those measurements which were difficult to make in the presence of Ca^{2+} ions were derived for acetate solutions in the presence of 40 mM Mg^{2+} by using in a number of cases large amounts of sucrose both outside and inside the muscle fiber. This was necessitated by the fact that the TMS theory required measurements in which the ratio of the two concentrations of K outside and inside was always constant. Under this stipulation, the values derived for the membrane potential in the barnacle muscle fiber ranged between -25.6 and -32.5 mV at pH 7.5 (Lakshminarayanaiah, 1974). This small change of about 7 mV for a change in the internal concentration of K from 100 to 600 mM always raised some doubts in one's mind about the reliability of using such data, however accurate that data might be, to derive values for the fixed-charge density in membranes. The fact that such data can be used to demonstrate a pattern of variation of $\bar{\phi}\bar{X}$ with change in the average ionic concentration has been recently illustrated (Lakshminarayanaiah, 1975*b*) by applying the K-theory. It is interesting to examine, in keeping with what has been accomplished above in the case of biomembranes of the squid nerve and frog muscle, the membrane potential data derived for the single fibers of the barnacle muscle internally perfused with potassium acetate solutions of different ionic strength maintaining a constant composition of artificial sea water outside.

These data reported in our earlier publication (Lakshminarayanaiah & Rojas, 1973) have been used to re-evaluate the effective charge density in the barnacle muscle membrane at pH 7.5. The experimental data and the results of the calculations are given in Table 8. In Table 9 are given also some data and calculations relating to the same barnacle preparation (*Megabalanus psittaccus*) in which the outside K concentration has been changed keeping the inside concentration of K constant. In this case it is seen at high K concentrations (also in some other Tables) that the measured values of membrane potential are larger than the values of E_{max}, the theoretically possible maximum value. This means that either the measurement is suspect (may be a nonsteady state measurement) or the chloride contribution to the membrane potential has increased. In either case no value for $\phi\overline{X}$ can be determined.

The values of $\phi\overline{X}$ given in Tables 8 and 9 show again an increase with increase in the average ambient concentration. The value of $\phi\overline{X}$ corresponding to the ionic media in which the barnacle muscle usually exists is around 0.17 M. Assuming a value of 0.2 for ϕ gives a value of 0.85 M for $\overline{X}$. This corresponds to a distribution of one negative charge per 195 $Å^2$ of membrane surface area.

The considerations and arguments presented above are based on the behavior of model ion exchange systems. On this basis the validity of all this exercise in simple arithmetic may be questioned by those who refuse to accept the extrapolation of model system analysis to biological membranes on grounds that biological membranes are thin and electrochemical theories are inapplicable to explain the voltage-dependent permeability characteristics of the biological membranes. The latter may be true; but what is demonstrated in this paper is not concerned with the excitability properties of the membrane but with its passive electromotive action which is governed not so much by its thickness but by the presence of ionogenic groups.

For purposes of comparision, the results given above and those existing in the literature for similar and/or other biological systems in which different experimental techniques have been used are collected in Table 10. The present potentiometric method described in this paper, unlike the methods based on measurements of conductance or threshold which are described to give values for the surface charge density near the ionic channels (Gilbert & Ehrenstein, 1969), gave an average value for the charge density present on the entire surface of the membrane. Results given in Table 10 show that the values obtained for the density of charges in various biological preparations vary from preparation to preparation. It

Table 10. Surface charges present in various biological membrane systems

Membrane system	Method used	One negative charge per N (number of Å^2) N	Reference
Muscle fibers, neurons in tissue culture	Application of electric field	2000	Elul (1967)
Fragmented sarcoplasmic reticulum	Electrophoretic	1000	Baskin (1972)
Squid axon	Electrophoretic	290 8000	Segal (1968) Gilbert (1971) using the results of Segal
Lobster axon	Electrophoretic	200 3500–4000	Segal (1968) Gilbert (1971) using the results of Segal
Squid axon	Curve fitting	700	Chandler, Hodgkin and Meves (1965)
	Membrane potential curve fitting	1600	Rojas and Atwater (1968)
	Conductance changes curve fitting	120	Gilbert and Ehrenstein (1969)
Rana node	Conductance change curve fitting	588	Mozhayeva and Naumov (1970, 1972)
Xenopus node	Conductance change curve fitting	291	Brismar (1973)
	Conductance change curve fitting	70	Vogel (1973)
Crayfish axon	Shift in threshold	43	D'Arrigo (1973)
Myxicola axon	Conductance change curve fitting	77	Schauf (1975)
Squid axon	Potentiometric extrapolation to the behavior of ion exchange membranes	222	present work
Frog muscle	Potentiometric as above	103	present work
Barnacle muscle	Potentiometric as above	195	present work

is surprising that even application of the same method of measurement (voltage shifts as a function of di- or trivalent ion concentration) to the

same biological preparation (*Xenopus* node) gave discordant values, and application of the same method to two different preparations (*Xenopus* node and *Myxicola* axon) gave agreeing values for the surface charge density. Another interesting feature of the results of Table 10 is that comparision of the result for the squid axon obtained by the present potentiometric method with that obtained by Gilbert and Ehrenstein (*see* Table 10) shows that the charge density near the ionic channels is greater than the average value derived for the whole membrane surface.

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