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Letter to the Editor

Addendum to: Potentiometric Estimation of Charges in Barnacle Muscle Fibers under Internal Perfusion*

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In the preceeding paper (Lakshminarayanaiah, 1975), it has been shown that a new potentiometric method due to Kobatake and colleagues is probably more reliable than a similar but old method due to Teorell, Meyer and Sievers (TMS) for estimating the effective charge density that is responsible for the electromotive action of membranes. These theoretical approaches due to TMS (Teorell, 1953; Meyer & Sievers, 1936) and Kobatake and colleagues (Kobatake & Kamo, 1973) are general and have been applied to estimate the concentration of fixed charges present in artificial ion exchange membranes which usually have thickness in the range 0.2–0.5 mm. The TMS theory, however, because of its existence for so long, has been applied to estimate the charges present in thin membranes of Parlodion ranging in thickness from 137 to 12,000 Å (Lakshminarayanaiah, 1966) and of bimolecular leaflet of lipid (oxidized cholesterol) of thickness 40 ± 10 Å (Tien, Carbone & Dawidowicz, 1966) doped with a protein material called excitability inducing material (EIM) (Latorre, Ehrenstein & Lecar, 1972). The important point is that these theories as applied for the evaluation of charge density of membranes are based on reliable measurements of membrane potential which is more dependent on the density of ionogenic groups present in the membrane than on its thickness. Barnacle muscle fiber membrane, like any other biological membrane, is thin and has ion exchange properties which have been very well documented (Hagiwara, Gruener, Hayashi, Sakata & Grinnell, 1968; Hagiwara, Toyama & Hayashi, 1971; DiPolo, 1972; Lakshminarayanaiah, 1974). So it is believed that the theory of Kobatake and colleagues which is more straightforward with fewer assumptions than the TMS theory (Lakshminarayanaiah, 1975) can be applied to describe the electromotive behavior of the barnacle muscle fiber membrane. Therefore, it is enlightening to see what kind of values are ob-

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Table 1. Density of various internal and external solutions used by Lakshminarayanaiah (1974, *see* Tables 1 and 2) to measure membrane potential E_m in internally perfused barnacle muscle fibers at pH 7.5 and 4.0

Solution no.	Internal	External
	pH = 7.5	
1	1.0899 ± 0.0001 [a]	1.0972 ± 0.0001
2	1.0786 ± 0.0003	1.0951 ± 0.0001
3	1.0691 ± 0.0002	1.0941 ± 0.0002
4	1.0557 ± 0.0001	1.0913 ± 0.0002
5	1.0290 ± 0.0003	1.0882 ± 0.0002
	pH = 4.0	
1	1.0847 ± 0.0002	1.0758 ± 0.0003
2	1.0811 ± 0.0001	1.0641 ± 0.0002
3	1.0729 ± 0.0003	1.0471 ± 0.0001
4	1.0670 ± 0.0001	1.0282 ± 0.0003

[a] Density (g/ml) ± 1 SE.

tained for the charges in the barnacle muscle fibers by applying this new method due to Kobatake and colleagues to the experimental data given by Lakshminarayanaiah (1974). To accomplish this, the concentration of K^+ (pH, 7.5) and Cl^- (pH, 4.0) ions need to be expressed on the molal scale. So the density of the various solutions used in the experiments by Lakshminarayanaiah have been determined and are given in Table 1. In calculating the molality M (moles per 1000 g water) of the solutions, appropriate weights of various ionic and nonionic species present at pH 7.5 and 4.0 have been taken into account. The values of M given in Tables 2 and 3 refer to the K^+ and Cl^- ion molalities, respectively.

Values given in Tables 2 and 3 for the other parameters were evaluated as follows:

$E_{\max}$ was calculated from the Nernst equation

$$E_{\max} = 58.16 \log (M_1/M_2). \qquad (1)$$

The values of $\bar{t}_{+(\mathrm{app})}$ given in Table 2 and of $\bar{t}_{-(\mathrm{app})}$ given in Table 3 were calculated from the equation

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

where E_m is the membrane potential measured (Lakshminarayanaiah, 1974).

Table 2. Evaluation of permselectivity P_s and effective fixed-charge density $\overline{\phi X}$ of the barnacle muscle fiber membrane at pH 7.5

M_2	M_1	E_m (mV) measured	E_{max} (mV) [Eq. (1)]	$\bar{t}_{+(app)}$ [Eq. (2)]	P_s [Eq. (3)]	$\xi = \frac{\sqrt{1-P_s^2}}{2P_s}$	$\overline{\phi X} = \frac{M_1+M_2}{2\xi}$
0.1172	0.0119	−32.5	−57.84	0.781	0.325	1.455	0.044
0.2303	0.0237	−31.8	−57.49	0.777	0.315	1.509	0.084
0.3383	0.0353	−30.6	−57.05	0.768	0.291	1.642	0.114
0.4388	0.0470	−27.9	−56.45	0.747	0.238	2.039	0.119
0.6287	0.0699	−25.6	−55.50	0.731	0.199	2.468	0.142

Table 3. Evaluation of permselectivity P_s and effective fixed-charge density $\overline{\phi X}$ of the barnacle muscle fiber membrane at pH 4.0

M_1	M_2	E_m (mV) measured	E_{max} (mV) [Eq. (1)]	$\bar{t}_{-(app)}$ [Eq. (2)]	P_s [Eq. (5)]	$\xi = \frac{\sqrt{1-P_s^2}}{2P_s}$	$\overline{\phi X} = \frac{M_1+M_2}{2\xi}$
0.1149	0.2244	−16.6	−16.91	0.991	0.939	0.183	0.927
0.1700	0.3253	−14.2	−16.40	0.933	0.603	0.661	0.375
0.2223	0.4168	−13.7	−15.88	0.931	0.593	0.679	0.471
0.2730	0.4989	−13.5	−15.22	0.943	0.656	0.575	0.671

Values for the permselectivity P_s given in Table 2 were calculated from the equation for a negatively charged membrane given in the preceeding paper (Lakshminarayanaiah, 1975), *viz.*

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

where t_+ is the transport number of cation in the aqueous phase. A value of 0.645 for t_+ derived from using the values of limiting mobilities for K^+ and Ac^- ions has been used in the calculations. It can be easily shown for a positively charged membrane that

$$\frac{1}{(1+4\xi^2)^{1/2}} = \frac{1 - t_+ - \bar{t}_{-(\mathrm{app})}}{t_+ - 1 + \bar{t}_{-(\mathrm{app})}(1 - 2t_+)} \tag{4}$$

where ξ is given by $[(M_1 + M_2)/2\,\overline{\phi X}]$, ϕ is a constant ($0 < \phi < 1$) and $\bar{X}$ is the stoichiometric concentration of charges (negative or positive) present in the membrane. $\overline{\phi X}$ has been called the effective fixed-charge density of the membrane. The permselectivity P_s is given by the right-hand side of Eq. (4), *viz.*

$$P_s = \frac{1 - t_+ - \bar{t}_{-(\mathrm{app})}}{t_+ - 1 + \bar{t}_{-(\mathrm{app})}(1 - 2t_+)}. \tag{5}$$

Using Eq. (5), values for P_s given in Table 3 were calculated. In these calculations the value of t_+ for the Tris cation $[(CH_2OH)_3CNH_3^+]$ used was 0.225. This is based on an assumption made regarding the Tris ion for which no value for its mobility seems to exist in the literature. Following Bezanilla, Caputo, Gonzalez-Serratos and Venosa (1972), it is assumed that the mobility of the Tris ion was half the mobility of β-hydroxyethyl ammonium ion $[HOCH_2CH_2NH_3^+]$; i.e., $2 \times 10^{-4}\ \mathrm{cm^2\ sec^{-1}\ V^{-1}}$ (Robinson & Stokes, 1959).

The values for the effective charge density $\overline{\phi X}$ given in the last column of Tables 2 and 3 show some interesting features. At pH 7.5, when the barnacle muscle fiber membrane is cation selective, the values of $\overline{\phi X}$ increase from 0.044 to 0.142 moles per 1000 g membrane water agreeing with the trend noted in the preceeding paper for well-characterized ion exchange membranes (Lakshminarayanaiah, 1975). However, these values are all higher than the average value of 0.019 M derived by the application of the TMS theory (Lakshminarayanaiah, 1974). At pH 4.0, when the fiber membrane is anion selective, the values of $\overline{\phi X}$, except for the value of 0.927, show a trend of increase from 0.375 to 0.671. Any lower value, like the two values of 0.044 and 0.084 observed at pH 7.5 is not noted at pH 4.0 probably because the average molality of solutions used at pH 4.0 is not that low. If all the values of $\phi\overline{X}$ at pH 4.0 are averaged, a value of 0.6 M is obtained. This value is slightly lower than the value of 0.7 M derived from the TMS theory. This average value of 0.6 M corresponds to a distribution of one positive charge per 277 $Å^2$ membrane surface. Similarly, taking the average of the three values at pH 7.5 corresponding roughly to the average concentrations of solutions at pH 4.0, i.e. a value of 0.125 M, a distribution of one negative charge per 1328 $Å^2$ membrane surface is obtained. These calculations are based on the assumption that the "pore" water is equated to a thickness of 10 Å (*see* Lakshminarayanaiah, 1974).

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