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Biological and Artificial Ion Exchangers: Electrical Measurements with Glass Microelectrodes

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Summary. Biological (*stratum corneum*) and artificial (cation-exchange resin beads, Bio-Rad AG 50W-X2) ion exchangers were impaled by glass microelectrodes filled with KCl solution. The electrical potential difference recorded in these structures in reference to the external bathing medium was shown to be dependent on the KCl concentration of both the external and the microelectrode filling solutions. The potentials were interpreted on the grounds of the fixed charge theory of membrane potentials as a consequence of two phase boundary potentials (Donnan potentials), one at the matrix-external solution interface and the other at the matrix-microelectrode solution interface. The contribution of a diffusion component for the recorded potential was considered.

Glass microelectrodes filled with electrolytic solutions have been extensively used to record intra and extracellular electrical potentials since the development of these microelectrodes by Ling and Gerard [12]. Under certain conditions, the electrical potential recorded by glass microelectrodes may be subject to errors due to the development of an emf, so called tip potential, at the level of the microelectrode tip. Reasons for the genesis of tip potentials are still ill defined [1, 2, 6, 19]. Tip potentials have been observed to depend on the nature and concentration of the filling solutions, electrode electrical resistance, ionic strength of the external solution, filling method, and several other variables [11, 15–18]. Under certain conditions, corrections can be introduced to take into account the tip potential value. Corrections of this kind can generally be carried out when the ionic characteristics (composition, ionic strength, pH, etc.) of the solution in which the microelectrode tip is immersed during the measurements are similar to that of the reference solution in which the tip potential is measured. On the other hand, when the physical-chemical properties of

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those solutions are different, errors, sometimes of significant magnitude, might be present and pass unnoticed by the investigator, unless he is aware of its occurence.

An important reason for the genesis of large emf's at the level of the microelectrode tip is its location within a fixed-charge bearing matrix, as is the case of cells of the *stratum corneum* [15] or the stroma of connective tissue [5]. The electrical potentials observed with 3 M KCl microelectrodes in the cells of the *stratum corneum* were interpreted as due to KCl diffusion from the microelectrode into the fixed-charge bearing protein matrix with K^+ and Cl^- transference numbers controlled by the matrix degree of protonation [10, 15]. The existence of intra-bead negative potentials was observed by Goldsmith *et al.* [8] with glass microelectrodes filled with 1 M KCl; this they interpreted as a Donnan potential jump at the bead-external solution interface. They also calculated single-ion activities of potassium ion in the resin phase. However, Cantwell and Saetre [4] questioned, purely on theoretical grounds, two fundamental claims of Goldsmith *et al.* [8]: (i) having measured an interfacial Donnan potential and (ii) having measured single-ion activities of potassium ion in the resin phase.

The aim of the present work was to make a comparative study of the results obtained in the *stratum corneum*, which is a biological ion-exchange matrix, and in beads of ion-exchange resin in order to interpret the nature of the electrical potential difference recorded in these structures with glass microelectrodes filled with KCl solution.

Materials and Methods

Glass microelectrodes were made from Kimax 51-Kimble glass tubing and filled with the desired solution by the glass fiber method [20]. Electrical potential difference between microelectrode and external solution was measured by an electrometer (Keithley, model 615) coupled to a recorder (Varian, model G-2500), via Ag-AgCl half-cell (connected to the microelectrode) and a saturated calomel half-cell connected to the bathing solution via a KCl salt bridge. Two different preparations were used: (i) Isolated skin of the toad *Bufo marinus ictericus*. Details of the method were published elsewhere [15]. In these experiments skins were bathed by KCl solutions on the outer side and NaCl-Ringer's solution on the inner side. The *stratum corneum* was impaled by microelectrodes which penetrated the skin from the outer side to 5 μm below the external surface. (ii) Cation-exchange resin beads (Bio-Rad AG50W-X2, 50–100 mesh size) were prepared according to Goldsmith *et al.* [8]. Beads in equilibrium with KCl solution of different molalities were impaled by microelectrodes. Penetration of the beads was carried out with no visual control, since the electrical behavior observed during penetration was identical to that described previously by Goldsmith *et al.* [8] under optical control. Electrical potential differences, $\Delta(\Delta\Psi)$, are the differences between

voltage measured within the *stratum corneum* (or the resin beads) minus that recorded with the microelectrode in the external bathing solution. Also, these values were steady-state values, normally attained a few seconds after impalement. Electrode resistances, measured in 2 M KCl, were within 2 and 35 Mohm. NaCl-Ringer's solution had the following composition (in mM): NaCl, 115; $KHCO_3$, 2.5; $CaCl_2$, 1.0; with pH of 8.2 after aeration. Results are presented as mean ±SEM. Straight lines were fitted by the least squares method.

Results and Discussion

Experiments were carried out in two different preparations (the isolated toad skin and beads of cation-exchange resin) in order to evaluate the mechanism of the genesis of the electrical potential difference recorded in these structures with glass microelectrodes, and the influence of the microelectrode KCl concentration on this potential difference. In the first group of experiments, toad skins were used. The impalement of the *stratum corneum* was carried out with a KCl external bathing solution of 0.1 M and NaCl-Ringer's solution as internal bathing solution. After a stable electrical potential difference was attained, the external solution was substituted by 0.01 M KCl. The hydrogen ion concentration of the bathing and of the microelectrode solutions were always identical and adjusted to pH 3 or pH 9 with HCl or KOH, without buffer. Table 1 summarizes the results. As can be seen, with external solutions more dilute than that of the microelectrode, $\Delta(\Delta\Psi)$ is significantly different from zero. However, when the concentration of the external solution is identical to that of the microelectrode, $\Delta(\Delta\Psi)$ is always zero. Analysis of Table 1 shows that $\Delta(\Delta\Psi)$

Table 1. Dependence of $\Delta(\Delta\Psi)$, recorded in the *stratum corneum*, on the composition of the external bathing solution and microelectrode filling solution

Electrode solution KCl	$\Delta(\Delta\Psi)$ (mV) External solution KCl	
	0.01 M	0.1 M
0.1 M – pH 9	−33.3 ± 1.9 (10)	0.2 ± 0.1 (10)
0.1 M – pH 3	32.6 ± 1.0 (16)	− 0.1 ± 0.1 (10)
3.0 M – pH 9	−82.1 ± 7.9 (10)	−29.7 ± 3.1 (10)
3.0 M – pH 3	49.2 ± 3.9 (10)	16.6 ± 1.8 (10)

$\Delta(\Delta\Psi)$ is the difference between the electrical potential difference recorded with the microelectrode within the *stratum corneum* (5 μm below the external surface) and that recorded with the microelectrode in the external solution. For all groups the microelectrode solution and the external solution had the same pH adjusted with HCl or KOH without buffer.

not only depends on the external solution concentration but also on the microelectrode filling solution. Thus, with external solution of 0.1 M KCl (pH 3 or pH 9), $\Delta(\Delta\Psi)$ is zero when the microelectrode filling solution is identical to the external bathing solution. However, $\Delta(\Delta\Psi)$ is no longer zero when the external solution is still 0.1 M KCl (pH 3 or pH 9) but the microelectrode solution is 3 M KCl. These results show that the microelectrode filling solution is an important parameter in determining $\Delta(\Delta\Psi)$. $\Delta(\Delta\Psi)$ in the *stratum corneum* has been considered to be a diffusion potential due to KCl diffusion from the microelectrode into the fixed-charge bearing matrix of the *stratum corneum* [10, 15]. Changes in $\Delta(\Delta\Psi)$ polarity with pH were related to alterations of K^+ and Cl^- transference numbers within the *stratum corneum*, due to changes in the degree of protonation of the matrix of this structure [10, 15]. According to this interpretation, when the microelectrode filling solution is identical to the external bathing solution, there is no driving force for KCl diffusion and, therefore, no reason for the genesis of an electrical potential difference. This, however, is not a unique interpretation, as will be discussed later.

The second group of experiments was carried out in cation-exchange beads impaled with glass microelectrodes filled with KCl of different concentrations. Fig. 1 shows the results of the experiments. Beads equilibrated with KCl of varying concentrations (in the range of 0.5 to 2000 mmolal) were penetrated sequentially by the same microelectrode. The slightest penetration of the beads caused an immediate jump in the recorded voltage. The behavior of our system, regarding electrical response during penetration, was very nearly the same as that described by Goldsmith *et al.* [8]. With 2 molal KCl filled microelectrodes it was observed that $\Delta(\Delta\Psi)$ was always negative as referred to the external solution (Fig. 1, open circles) and a linear function, in the range of 0.5 to 100 mmolal concentration, of the mean molal activity of KCl ($a_\pm = m\gamma_\pm$, where m = KCl molality and $\gamma_\pm$ = mean activity coefficient) in the external medium. Above 500 mmolal, deviation from linearity is observed. Deviation from linearity was also observed by Goldsmith *et al.* [8] at high KCl concentrations. These authors interpreted the observed potential difference as a result of a Donnan potential at the bead-external solution interface. They considered that the ion-exchange beads in solution are largely an aqueous phase, and they assumed the liquid junction potentials between microelectrode and the ion-exchange matrix to be those arising in simple KCl solutions of equivalent ionic strength. Under these conditions they expected the junction potential errors to be negligible. Bearing in mind the results with toad skins, described

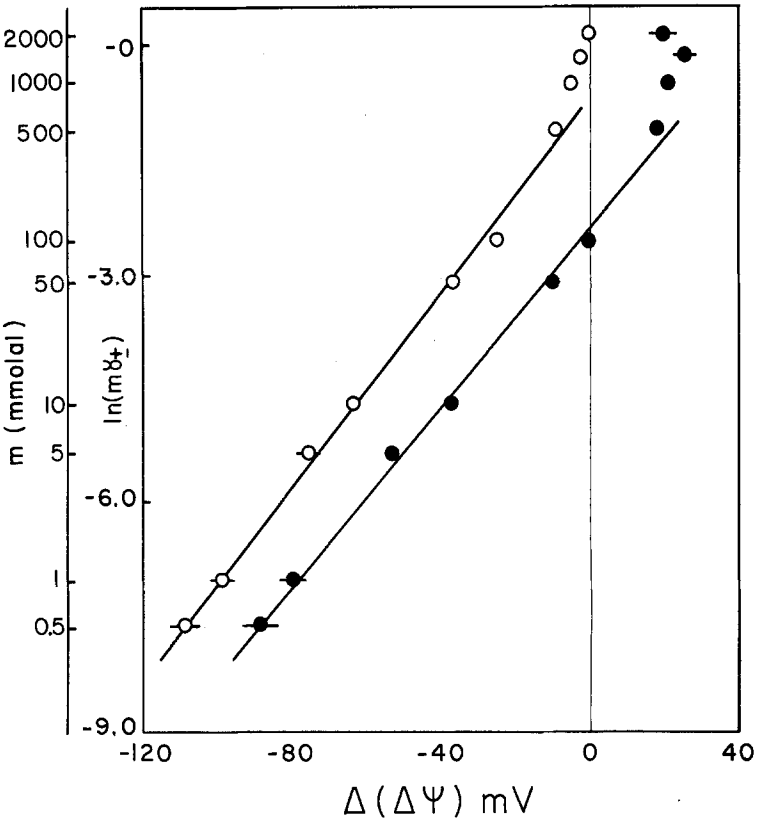

Fig. 1. Dependence of $\Delta(\Delta\Psi)$ (recorded in cation-exchange resin beads with microelectrodes of two different compositions) on the composition of the external bathing solution. $\Delta(\Delta\Psi)$ is the difference between the electrical potential difference recorded with the microelectrode within the resin bead and that recorded with the microelectrode in the external solution. m is the KCl molality of the external solution. $\gamma_{\pm}$ is the KCl mean ionic activity coefficient. Open circles correspond to microelectrodes filled with 2000 mmolal KCl solution and closed circles to microelectrodes filled with 100 mmolal KCl solution

earlier in this paper, experiments were planned and carried out to verify the possibility of a similar behavior in cation-exchange resin beads. In Fig. 1, closed circles, is shown the results obtained with 100 mmolal KCl filled microelectrodes in the same bead populations impaled by the microelectrodes filled with 2000 mmolal KCl solution. As can be seen, the dependence of $\Delta(\Delta\Psi)$ on $a_{\pm}$ is similar to that observed with 2000 mmolal KCl filled microelectrodes. However, with electrodes filled with more dilute solutions, $\Delta(\Delta\Psi)$ values are shifted to the right, both lines being almost parallel. Also, $\Delta(\Delta\Psi)$ is zero when the microelectrode solution is identical to the bathing solution and reverses its polarity when the external solution is

Table 2. $\Delta(\Delta\Psi)$ recorded in cation-exchange resin beads with external solution and microelectrode solution having the same composition

KCl solution (mmolal)	$\Delta(\Delta\Psi)$ (mV)
0.1	-0.1 ± 0.2
1	0.2 ± 0.1
10	-0.1 ± 0.1
100	0.0 ± 0.1
1000	-0.1 ± 0.1
2000	0.0 ± 0.0

$\Delta(\Delta\Psi)$ is the mean of 20 penetrations.

$\Delta(\Delta\Psi)$ is the difference between the electrical potential difference recorded with the microelectrode within the resin bead and that recorded with the microelectrode in the external solution.

made more concentrated than that of the electrode. The slope of $\Delta(\Delta\Psi)$ as a function of $\ln a_{\pm}$ was 37 mV per decade with 2000 mmolal KCl microelectrodes and 40 mV per decade with 100 mmolal KCl microelectrodes. These values are lower than the Nernstian slope observed by Goldsmith *et al.* [8]. Table 2 completes the picture, showing that in the range of 0.1 to 2000 mmolal KCl, $\Delta(\Delta\Psi)$ is always zero when the external solution and microelectrode solution are identical. These experiments, with those of Fig. 1, clearly show that $\Delta(\Delta\Psi)$ cannot be interpreted as being due to a single Donnan potential jump at the bead-external solution interface, as proposed by Goldsmith *et al.* [8], since the results show that with the same external solution, $\Delta(\Delta\Psi)$ varies with the electrode solution. Therefore, the dependence of $\Delta(\Delta\Psi)$ on the microelectrode KCl concentration should be the same as that on the external medium KCl concentration. This implies that the measurements with microelectrodes in ion-exchange resins should not provide, as emphasized by Cantwell and Saetre [4], access to the internal phase of the ion-exchange matrix, as suggested by Goldsmith *et al.* [8] and Goldsmith [7].

The results of the present experiments can be interpreted by following the tenets of the fixed charge theory of membrane potentials proposed by Teorell [21, 22, 23] and Meyer and Sievers [14]. Thus, the potential can be assumed to be composed of two phase boundary potentials (Donnan potentials), one at the external solution-matrix interface and the other at the microelectrode solution-matrix interface, and of a diffusion potential between the two interfaces. For the *stratum corneum*, Nunes and Lacaz Vieira [15] and Lacaz Vieira *et al.* [10] tentatively interpreted the results on

the basis of the diffusion component alone due to its relatively low ion permselectivity, which is controlled by the matrix degree of protonation. However, asymmetric phase boundary potentials can also play a role on the observed electrical potential measured in the *stratum corneum.*

The same approach can be used to treat the results obtained in ion-exchange resin beads as suggested by Cantwell and Saetre [4]. The results of Fig. 1 can be interpreted by assuming that the shift of the line to the right, which follows reduction of microelectrode KCl concentration, as being due to changes in the phase boundary potential at the microelectrode-resin interface. When the electrochemical cell is symmetric (external solution identical to microelectrode solution; Fig. 1 and Table 2), $\Delta(\Delta\Psi)$ is always zero since the two phase boundary potentials cancel each other and the diffusion component is also zero. The non-Nernstian slope observed in Fig. 1, 37 and 40 mV per decade, respectively, for 100 and 2000 mmolal microelectrodes, may indicate that the resin is not perfectly cation permselective [9]. However, it is conceivable that this deviation could also arise as a consequence of the system not obeying the fundamental assumption of the theory of membrane potential [21, 22, 23] that the counterions are free within the resin matrix. There are in the literature evidences in favor of this possibility, which suggest that the counterions behave as being mostly adsorbed to the polyelectrolyte matrix [3, 13].

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