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Letters to the Editors

Microelectrode Artifacts and Frog Skin Potentials

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From microelectrode studies on intact and cryo-destroyed frog skins, Nelson, Ehrenfeld and Lindemann [5] point out the necessity to "re-investigate the membrane potentials of amphibian skin epithelium with more suitable techniques." The authors apparently missed that previously published measurements of potentials comprise two groups – those before and after 1975 (for reference *see* [5]) – with completely different potential values and profiles, as shown in Fig. 1 *a* and *b* schematically. In view of the evident and considerable differences between the two groups of previously reported microelectrode studies it would be highly confusing and unreasonable to discuss effects of artifacts in common for both.

Presumed artifactual "pre-tip-potentials" of about -20 mV[1] were *always* generated when microelectrodes are inserted into the frog skin (or other epithelia), intracellular potentials as reported between 1957 and 1973 (-10 to -35 mV under short-circuit conditions) would, on the average, be about zero millivolts. Missing response of these potentials upon functional variations of the epithelium would be consistent with such artifactual origin. In contrast, "pre-tip-potentials" of the same magnitude would reduce intracellular potentials (short circuited, no transcellular current flow) from studies after 1975 to values of -80 to -100 mV. Alterations of the intracellular potentials, related to the functional state, would not be affected at all!

KCl loss from 3-M KCl electrodes with consecutive swelling and damage of the cells is concluded by Nelson *et al.* from microscopic observation during impalement of the frog skin. The authors' unique technique for microelectrode impalement (Fig. 3 "after impalement ... the electrode was slightly withdrawn ..."), however, must be suspected to result in injury of the cell itself [4]. Thus, even the initial values are presumably recorded from injured cells. Use of 3-M KCl electrodes may accelerate the eventual complete osmotical damage. Nevertheless, *all* (initial and final stable) readings would then represent "some mean of the potentials of the two bathing solutions" [5], as suggested for results of studies before 1975 [4, 1].

Nelson *et al.* [5] restrict their discussion regarding the period *prior to swelling* on "pre-tip-potentials" resulting from suspected negative fixed charges and KCl loss from the microelectrode. If present, these artifacts account for -10 to -25 mV under worst conditions. KCl loss from the microelectrode, on the other hand, may increase the intracellular [K] and, from this, hyperpolarize the basolateral membrane potential above the normal value. Artifacts of this type are highly unlikely since:

1) The intracellular potential may assume a steady value immediately after impalement (100 msec) and remain unchanged for periods of several minutes or hours (e.g., *see* Fig. 3 of [1]).

2) The intracellular potentials are independent of the microelectrode resistance (and amount of KCl loss) before and during impalement [4].

[1] These high "pre-tip-potentials" were observed [5] when "electrode tip pressure on protoplasmic constituents was (purposely) maintained during recording." When this was not intended, considerably lower "pre-tip-potentials" were obtained.

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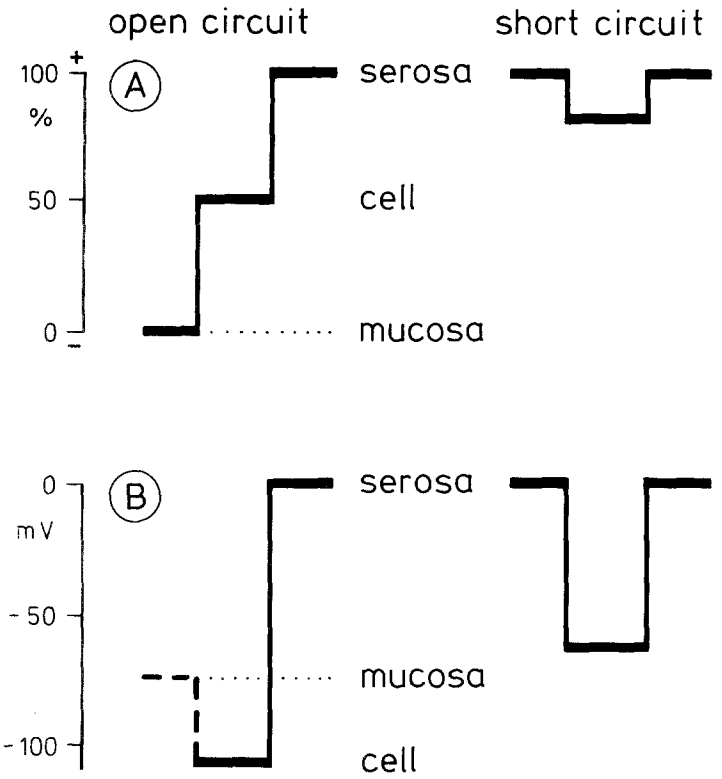

Fig. 1. Potential profile of amphibian skin epithelium under open-circuit and short-circuit conditions. (*A*): Data as reported before 1975. The potential scale is given in % of the transepithelial potential. PD is −20 mV under short-circuit conditions. (*B*): Values from studies after 1975. The potential difference between mucosal solution and cell is variable

3) Increase of the intracellular [K] (presumably some 100–120 mM from osmotical reasons) to 140 mM would change the potential by 6 mV only, if the cell remained intact.

4) Intracellular potentials were identical whether microelectrodes filled with 3 M KCl, 0.15 M KCl, or other electrolyte solutions (4 M K-acetate, 3 M NH_4NO_3) were used [2].

5) Measurements of K loss from microelectrodes [2, 3] allow us to calculate that Nelson *et al.* [5] overestimate the amount of K loss considerably.

It appears that "more suitable techniques" [5] have been applied intuitively in studies on intracellular potentials of frog skin since 1975. Further improvement may be possible. Discussion of microelectrode artifacts should, on the other hand, not be restricted to data from amphibian skins. Critical and unbiased reinvestigation of potential profiles of other epithelia may reveal presently unexpected errors from various types of artifacts and provide realistic description of the electrophysiological behavior of the individual epithelial membranes.

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Reply to: Microelectrode Artifacts and Frog Skin Potentials

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In response to the recent letter by Wolfram Nagel [5], I should like to add the following comments. The paper of Nelson *et al.* [6], as will be apparent from its title, is not primarily concerned with the question, which of the different potential profiles previously reported for frog skin epithelium comes closest to the truth. The quoted papers – irrespective of the potential profiles reported in them – were discussed together to the extent in which they recommend the use of low-resistance micropipettes filled with 3 M KCl. None of them, before or after 1975, shows awareness of the two problems which we have described. A partial exception is merely Nunes and Lacaz Vieira [7], who recognized the pretip potentials but underestimated the KCl release.

There seems to be agreement that a pretip-potential would contribute an unknown additive quantity of up to -20 mV to the measured potential. Where such accuracy is not sufficient, more suitable methods will necessarily have to be developed. On the other hand, where an offset of 10 to 20 mV can be disregarded and KCl release from the tip does not cause additional problems, Nagel's present method will be usable and will presumably yield many interesting results.

The impalement technique of Helman and Fisher [1] and of Nagel [4] is essentially a stepwise advance of the micropipette tip from the outside through the corneum into deeper layers of cells. Thereby the tissue is dimpled and exerts a force on the shaft of the pipette as well as, presumably, on the tip. Thus, organic material may well be pressed against the tip and tissue elasticity may keep the pressure up, even when the pipette is slightly withdrawn. We have shown that in such situations a negative pretip-potential may continuously be recorded as long as the density of negative fixed charges in the cell interior is not reduced. While the pretip-potential is noticeable, KCl-release from the tip may be more or less suppressed [6].

If on slight withdrawal of the micropipette a decrease in negativity is observed, this need not (or not alone) be caused by a re-opening of the impalement shunt [3]. Rather, since the phenomenon is also found in epithelia with destructed membranes, the diminution

of a pretip-potential may contribute to or completely explain the decrease in negativity [6].

We expect that in those cases where the pretip-potential becomes smaller on withdrawal, KCl-release will increase. It appears, therefore, that when using pipettes of low resistance filled with 3 M KCl one will encounter either Scylla (offset) or Charybdis (release) depending on tip pressure. It may be possible to plot a course between these hazards, but to do this it helps to know that Scylla and Charybdis exist, and this was our message.

The rate of KCl release from unobstructed tips has recently been measured by Gerrit Isenberg [2]. According to his Fig. 9, the diffusional rate is only about 15% of those calculated maximal values which we quoted from the literature. Isenberg was the first who managed to estimate these rates experimentally. Incidentally, his paper contains an error which came about by taking the ratio instead of the difference of concentrations to calculate the diffusional gradient. Thus, the rate of release, while given correctly in Fig. 9, is about 1,000-fold larger than stipulated on page 96 (lower right-hand corner) of reference 2. It appears that an unobstructed tip of 5 MΩ resistance will release 10^{-14} and a tip of 25 MΩ about 2×10^{-15} osmoles per second (extrapolated) by diffusion. This is rather much for a cell of less than 1 picoliter volume containing initially for example 10^{-13} osmoles, and quite sufficient to explain the cellular swelling which we observed on impalement.

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