Changes in Ionic Conductances and in Sensitivity to Amiloride during the Natural Moulting Cycle of Toad Skin (*Bufo viridis*, L.)

Uri Katz

Department of Zoology, The Hebrew University of Jerusalem, Israel

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**Summary.** The resistance of the apical membranes of toad skin (*Bufo viridis*) was measured during its natural moulting cycle using a fast flow technique. The skin behaved in all periods of the moulting cycle as a nearly perfect sodium electrode. In the presence of amiloride (10^{-4} M), the total resistance of the same skin was identical with solutions which contained either sodium or potassium. The resistance of the skin with potassium was sensitive to amiloride in the period just after moulting. The resistance of skins which were made shunted by treating them with urea on the outside was insensitive to amiloride in solutions containing potassium; a small effect was still observed with sodium. It is suggested that the transient sensitivity to amiloride, with potassium, is the result of differentiation of the sodium specific sites at the apical membranes of the skin.

Shedding of the *stratum corneum* of the skin in toads and frogs is accompanied with considerable changes in physiological characteristics of the skin (Jorgensen, 1949; Nielsen, 1969; Hviid-Larsen, 1970; and others). The increase in short-circuit current following slough formation was accounted for by an increase in the rate of active sodium transport and an increase in the passive permeability to sodium (Hviid-Larsen, 1970).

The fast flow technique, which has been developed by Lindemann and co-workers (1972), allows a direct study of the apical functional membranes of the skin by recording electrical changes while rapidly changing solutions over it. This technique has been applied for a study of the characteristics of passive permeability of the outer barrier of toad skin during its natural moulting cycle.

**Materials and Methods**

Toads, *Bufo viridis*, were collected in Israel. Animals of both sexes were kept separately at 21 ± 2 °C in deionized water (2–3 cm deep), and were not fed during the experimental
Fig. 1. Instantaneous $R-c$ curves (resistance as a function of concentration) of toad skin epithelium, during the natural moulting cycle. Period 1: time when slough just came off; period 2: 3–5 hours later; period 3: time in between two moults. (A): the resistance measured in solutions with increasing concentrations of sodium; (B): the resistance measured in solutions with increasing concentrations of potassium. Open circles represent determinations which were taken in the presence of $10^{-4}$ M amiloride. Skins were preequilibrated with 1 mm Ca-gluconate, 5 mm Tris-sulfate, pH = 6.0, on the outside; and sulfate-agar Ringer's on the inside.

period. Shedding was followed by inspecting red lipstick marks on the neck of the animals, which disappeared upon shedding. The toads moulted about every 4–5 days under these conditions at regular intervals. Animals were picked up at a desired timing along their individual moulting cycles after 7–8 successive observed moults. They were double pithed, and the abdominal skin was dissected and mounted in the ‘fast flow’ apparatus, as described by Lindemann et al. (1972). In brief, an area of 1 cm$^2$ of the skin formed in the apparatus the bottom of a shallow flow channel (1 mm high). Solutions which flow through the