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WATER MOVEMENT ACROSS SPLIT FROG SKIN

YING T. LAU, ROBERT H. PARSONS, ROBERT J. BRADY and GAIL A. FEENEY
Department of Biology, Rensselaer Polytechnic Institute, Troy, NY 12181 (U.S.A.)
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Summary

A net inward fluid reabsorption (salt-linked flow) has been observed in isolated skin epithelium (split skin) with the same magnitude as in whole skin when identical NaCl Ringer solutions were used to bathe both sides. Split skins also respond to a hyperosmotic sucrose solution bathing the outer (epithelial) surface by generating an outward osmotic flow. A non-linear relationship between osmotic flow and the osmotic gradient has been found in split skin similar to that found in whole skin.

Introduction

Frog skin epithelium separated from the corium (split skin) has a similar general morphology to that of epithelium in situ except for the stratum germinativum cells [1,2]. The intercellular junctions and spaces are comparable to those seen in the epithelium in whole skin [2]. The split skin also maintains Na^+ transport characteristics (short circuit current and potential difference) as well as a responsiveness to ouabain, 2,4-dinitrophenol, oxytocin, and vasopressin [2—4]. Although the epithelial cells represent less than one-fifth of the total skin thickness, the osmotic water permeability and its responsiveness to oxytocin, cyclic AMP and theophylline are the same as in whole skin [5,6]. Largely due to the difficulty in preparing large sheets of split epithelium needed for water flow studies the salt-linked water flow, i.e. a net inward transepithelial fluid movement without any apparent net driving force has not been investigated in the split skin preparation. In addition the osmotic flow-osmotic gradient relationship has not been determined. This study, using the technique of Hoshiko and Parsons [7] to produce large sheets of split skin, investigates both salt-linked water flow and osmotic water flow across split and whole frog skin. This allows an evaluation of the role of the corium in these processes.

Materials and Methods

Animals. Rana pipiens, obtained from J.M. Hazen in Vermont were kept in running tap water with free access to a dry area. To ensure uniform hydration, the frogs were placed in 6 inch of tap water for 36—48 h at 15° C before use. Animals were killed by double pithing and abdominal skin was removed and cleaned of all adhering muscle and connective tissue. The isolated epidermis (split skin) was then obtained with trypsin treatment by the method of Hoshiko and Parsons [7].

Experimental protocol. Four sets of chambers were employed, two split skins and two whole skins were studied on the same day with the same protocol. The tissue was mounted with identical NaCl Ringer solutions on both sides (220 mosM, 112.4 mM Na^+, 2 mM K^+, 1.8 mM Ca^{2+}, 115.6 mM Cl^-, 2.4 mM HCO_3^-, pH 7.8).

The volume flow apparatus was similar to that used by Franz and Van Bruggen [8]. The skin was placed between two lucite half-chambers. One of the chambers was sealed off except for a small outlet capillary connected to a microliter buret assembly with digital read-out. When a reading was taken a small pressure was applied to the opposite chamber forcing the skin against a stiff nylon screen located between the chambers. This assured a fixed skin position for each reading. The fluid level in the outlet capillary was brought to a reference point by means of the microliter buret. The volume needed to make this adjustment was the volume which had moved across the skin (precision ± 0.15 μl). The volume flow was measured at 20-min intervals for 1 h. The rate of water flow during the last two intervals was taken as the salt-linked flow. At the end of the first hour, the epithelial (E) bath was changed to a NaCl Ringers solution with sucrose added to make it hyperosmotic to the corium (C) bath. Four different sucrose concentrations were used which gave final osmotic gradients of 100, 340, 500 and 700 mosM. The osmolarities of all solutions were measured on an Osmette Precision Osmometer (precision ± 1.8 mosM). Volume flow was then measured for 20-min intervals for an additional hour. Only the last two intervals were used to determine the volume flow. To obtain the actual osmotic flow the salt-linked flow was added to the measured volume flow. Addition is required because salt-linked flow and the osmotic flow in these experiments are in opposite directions. In a few experiments NaCl Ringer solution containing 1 mM KCN or an isotonic Na_2SO_4 Ringer solution (50 mM Na_2SO_4 and 50 mM sucrose) replaced the standard Ringers solution. In these experiments, tissues were immersed in the same solution (KCN or Na_2SO_4) at least 1 h before mounting on the chambers.

Mannitol permeability was determined by adding [^{14}C]mannitol to the epithelial facing bath after 2 h. A sample was also taken from the epithelial facing bath at this time. The samples were added to Triton X-100 counting cocktail [9] and counted to 1.5% error using a Liquid Scintillation Counter (Beckman LS-230). An external standard was used to determine quenching. The [^{14}C]mannitol permeability was then determined as follows: $P_M = \Delta C \cdot \Delta T^{-1} \cdot \mathrm{TBA}^{-1} \cdot A^{-1}$. Where ΔC is the amount of counts (cpm) appearing during the time interval ΔT (s) in the corium facing bath, TBA is the total bath activity per ml (cpm/cm^{-3}) in the epithelial facing bath and A is the area (3.80 cm^2), P_M

is then the mannitol permeability in cm/s. There was no significant difference among tissues exposed to different osmotic gradients thus the mannitol permeabilities are the average pooled values from all four gradients tested.

All values in this paper are given as mean ± S.E. (number of skins).

Results

A net volume flow from the epithelial bath to the corium bath is measured in the absence of an osmotic gradient. This salt-linked flow is observed with both split and whole skins. In split skin preparations, the salt-linked flow has an average of 3.08 ± 0.58 (16) μl/cm^2 per h which is significantly different from zero ($P < 0.001$) but is not different from that observed in whole skin: 3.23 ± 0.50 (24) μl/cm^2 per h. The salt-linked flow can be diminished by KCN or Na_2SO_4 Ringer solutions. The net volume flow for the last measurement in the first hour is 0.01 ± 0.12 (3) and 0.07 ± 0.10 (3) μl/cm^2 per h for split skin bathed in KCN and Na_2SO_4 Ringer solutions, respectively. Similar results have been obtained for whole skins [10]. However, even bathed in the normal NaCl Ringer solution, split skin shows a decrease of the salt-linked flow with time (42% drop for 1 h) which is more pronounced than that of whole skin (14% drop for 1 h). This decrease was also observed by House [10] in whole skin despite the maintenance of stable electrical potentials.

After sucrose is added to the epithelial bath to create an osmotic gradient, a net volume flow from the corium to the epithelial bath is observed for both split and whole skins. However, as the sucrose concentration, which equals the transepithelial osmotic gradient, increases, the osmotic flow (sum of observed volume flow and salt-linked flow) does not increase proportionally. As the osmotic gradient increases, the osmotic permeability (L_p) decreases (Table I).

The [^{14}C]mannitol permeability of 1.07 ± 0.20 · 10^{-6} cm/s ($N = 10$) for split skins is not significantly different from that for whole skins of 1.91 ± 0.43 · 10^{-6} cm/s ($N = 8$), indicating that removal of the corium in the split skin preparation does not change the permeability to a relatively large non-electrolyte.

TABLE I

DEPENDENCE OF OSMOTIC PERMEABILITY (L_p) ON HYPEROSMOTIC GRADIENT (ΔC)

ΔC (mosM)	L_p (μl/cm^2 per h per 100 mosM)	
	Split skin	Whole skin
100	4.45 ± 0.97 (4)	7.32 ± 2.17 (6)
340	2.46 ± 0.54 (4)	3.32 ± 0.65 (6)
500	2.17 ± 0.36 (4)	3.26 ± 0.37 (6)
700	1.46 ± 0.26 (4)	2.28 ± 0.29 (6)
P (100—700)	<0.025	<0.05

Discussion

The results in this paper demonstrate that in the absence of a trans-tissue osmotic gradient, a net trans-tissue reabsorption exists in the isolated skin epithelium (split skin) and is quantitatively the same as that observed in whole skin. The salt-linked flow can be inhibited by 1 mM KCN or by substituting Na_2SO_4 for NaCl in the bathing solutions. It has been reported that cyanide and sulfate Ringer solutions [10] inhibit salt-linked flow in whole skin from *Rana temporaria.* Thus it appears that the salt-linked flow observed in split skin is very similar to that observed in whole skin, and the corium (connective tissue layer) is not required for this process.

House [10] has proposed a double-membrane model to explain the formation of salt-linked flow in frog skin. If the outer membrane is permeable to Na^+ but not to K^+ and if the inner membrane has opposite permeability properties, a net transepithelial fluid movement would be expected in the steady state. Our results suggest that both membranes are located within the epithelium cell layers since salt-linked flow exists in the absence of the corium. However, it cannot be asserted which is the middle compartment involved, epithelial cells, intercellular channels, or both.

Osmotic experiments were performed and our results clearly show a non-linear relation between osmotic flow and osmotic gradient in both split and whole skin. As the osmotic gradient increases, the osmotic permeability (L_p) decreases. The present results are consistent with the view that non-linear osmosis is a property mainly of the epithelium cell layers since non-linearity exists in split skin preparations as well as in whole skin. Rajerison et al. [5] reported an osmotic permeability of 8.2 $\mu l/cm^2$ per h per 100 mosM for *R. esculenta* L. split skin when the epithelial side was bathed with a 1/20-diluted Ringer solution. This value is somewhat higher than our results. Since a hypo-osmotic solution was used in their experiment, the discrepancy may be due to an effect of the hyperosmotic bath or it could be due to a species difference.

Thus in addition to electrical properties and responsiveness to hormones split-frog skin shows a water movement similar to that found in whole frog skin. This indicates that the corium plays little if any role in the mechanism responsible for salt-linked water flow or in the mechanism responsible for non-linear osmosis.

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