UREA PERMEABILITY IN PREGNANT ENDOMETRIUM OF SPINY DOGFISH (SQUALUS ACANTHIAS)

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The concentration gradient from blood to uterine fluid for urea (about 315 mM) across the endometrium of Squalus acanthias in pregnancy suggests that this epithelium may be relatively impermeable to urea. Studies were undertaken to measure the permeability of urea and the mechanisms by which it takes place.

Pregnant uteri were removed from freshly sacrificed S. acanthias and placed in ice-cold dogfish Ringer's ($NaCl$ 280, KCl 6, $CaCl_2$ 5, $NaHCO_3$ 8, NaH_2PO_4 1, $MgCl_2$ 3, Na_2SO_4 0.5, urea 350 and glucose 5 mM, pH - 7.6). The endometrium was stripped from the underlying tissues and tied over glass bungs. Permeability was measured by placing the tissue in a 35 ml volume of dogfish Ringers with labeled solute (0.5 to 1.0 μci/ml). The volume of the unlabeled side was 2.5 ml dogfish ringers and both sides were stirred with magnetic bars (Hays, R.M. et al in Urea and the Kidney Ed. B. Schmidt-Nielsen, Excerpta Medica, Amsterdam, 1970). The area of membrane exposed was 3.14 cm^2. Experiments were carried out at 15°C and permeability was measured by removing 50 μl samples from the recipient side at 30-60 minute intervals, after allowing 60 minutes to reach steady state conditions.

Table 1 gives the results for water (^3HOH), urea, D-glucose and mannitol (5mM). The small difference e between glucose and mannitol suggests that there is no facilitated pathway for sugar transport in this epithelium. The ratio of P urea/P mannitol (8-9) exceeds the ratio of their free solution diffusion coefficients which is about 2. The usual explanation for this finding is that these hydrophilic nonelectrolytes permeate through an aqueous channel which offers steric hindrance to the larger solute. Other possibilities however exist. Based on the observation that phloretin

Table 1. Solute permeability in dogfish endometrium

Solute	Number of Experiments	Permeability ($cm.sec^{-1} \times 10^6$) S → M	M → S
Water	5	35.24 ± 2.13	36.50 ± 4.63
Urea	5	0.58 ± 0.07	0.65 ± 0.09
D-Glucose	4	0.160 ± 0.005	0.123 ± 0.016
Mannitol	4	0.064 ± 0.010	0.078 ± 0.018

Values are mean ± SEM. S →M represents experiments performed with labeled solute placed at serosal surface and M → S at mucosal surface.

($1\text{-}5 \times 10^{-4}$M) causes a 50-8- percent decrease in urea permeability it has been proposed that its permeability is in part facilitated in the erythrocyte (Macey and Farmer, Biochim. Biophys. Acta. 211: 104, 1970), toad bladder (Levine et al. J. Clin. Invest. 52: 1435, 1973) and rabbit gallbladder (van Os et al J. Memb. Biol. 15: 363, 1974).

In a second series of experiments, following control measurements over 120 minutes, phloretin in ethanol was added to the ^{14}C-urea donor side to bring the phloretin concentration to 10^{-4}M and the ethanol concentration to 0.5 percent. The experiments were carried out at pH 7.4 and phloretin was always added to the mucosal side. A control matched piece of endometrium had only the ethanol (0.5%) added to the mucosal solution. The results which are given in Table 2 indicate that phloretin has no effect on urea transport in this tissue.

Moreno(J. Gen. Physiol. 66: 117, 1975) has proposed two paracellular pathways (in addition to a cellular route)

Table 2. Effect of phloretin on urea permeability in dogfish endometrium

	Control	Ethanol (0.5%)	Control	Phloretin (1×10^{-4}M)
P urea ($cm.sec^{-1} \times 10^{6}$)	0.53 ±0.04	0.45 ±0.05	0.53 ±0.07	0.61 ±0.07

Values are mean + SEM for 5 experiments. Labeled urea placed at the mucosal surface for all experiments. Following the control period phloretin in ethanol placed at the mucosal surface to bring the concentration to 1×10^{-4}M and 0.5% ethanol. Matched tissue had only 0.5% ethanol placed at the mucosal surface.

for small nonelectrolyte permeation in gallbladder of frog and rabbit. In one of these, the cation selective pathway, 2,4,6-triaminopyrimidine (TAP) blocks urea transport. In separate studies carried out with both the mucosal and serosal solutions at pH 6.4 we examined the effect of TAP on urea permeability. The protocol was similar to that for the phloretin experiments. TAP (10 mM) was added to the mucosal solution and mannitol (10mM) to the serosal solution. Mannitol (10mM) was added to both sides of the matched control tissue. Urea permeability was measured from mucosa to serosa. The results are given in Table 3. The 36 percent inhibition in urea permeability is similar to that reported for rabbit gallbladder (44 percent) but less than the 60 percent observed in the frog (Moreno, J. Gen. Physiol. 66: 117, 1975). The specificity of this effect would await experiments carried out with water and mannitol as the permeants.

Table 3. Effect of Triaminopyrimidine (tap) on urea permeability in dogfish endometrium

	Control	Mannitol (10mM)	Control	Tap (10mM)
P urea ($cm.sec^{-1} \times 10^{6}$)	0.57 ±0.09	0.53 ±0.06	0.58 ±0.06	0.37 ±0.07

Values are mean + SEM for 6 experiments. Labeled urea placed at mucosal surface for all experiments. Following the control period tap 10 mM placed at mucosal surface. Matched tissue had mannitol 10 mM placed at mucosal surface and both tissues had mannitol 10 mM placed at serosal surface. Control and addition experiments carried out at pH 6.4.

In an effort to estimate the extent of urea entry at the mucosal face of the endometrial epithelial cell we measured ^{14}C urea uptake in tissue mounted as for the permeability studies but exposed to the labeled solution for only 45 sec. Correction for extracellular space was made with ^{14}C-polyethyleneglycol (MW = 4,000) (PEG). With the mucosal surface presented to the labeled substances the volume of distribution for PEG measured at 45 sec. (2.41 ± 0.24 µl/100 mg tissue wet wt.) was only slightly less than that measured after 1 hour of exposure (3.09 ± 0.19). However, with the serosal aspect of the epithelium exposed the distribution at 1 hour 26.8 ± 1.7 µl/100 mg far exceeded that at 45 sec (4.94 ± 0.52), so that the method could only be applied to determine mucosal permeability. Urea permeability of the mucosal epithelium was 25.4 ± 2.6 cm $sec^{-1} \times 10^{-6}$ (n = 3). This value which is forty times that for permeability across the entire epithelium (Table 1) suggests that the major barrier to transcellular urea transport is at the serosal aspect of the endometrial epithelium. Supported by grants from NIH-NICHD (HD00139 and HD12033) and NIEHS (ES01678).