# MEASUREMENT OF WATER, UREA AND OXYGEN PERMEABILITIES IN EEL SKIN, AND SWIM BLADDER AND FLOUNDER OPERCULUM.

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Since both summer flounder and eel can exist in fresh or salt water, they must be capable of maintaining osmotic gradients across the skin (eel) or gills/operculum (flounder). Fom these considerations, water and small nonelectrolyte permeabilities across eel skin and flounder operculum should be low. By contrast, because eels can exist out of water and because of the respiratory function of gill and operculum, we anticipate tha oxygen permeabilities of these structures should be relatively high, especially when compared with those of the eel swim bladder, a structure which is adapted to trap oxygen under high pressure, depending on the depth to which the eel is adapted.

For these studies the summer flounder *Paralichthys denatus* and eel (*Anguilla rostrata)* were used. Both fish were kept in sea water conditions. The flounder were removed from the tank, iced and then pithed by transection of the spinal cord behind the head. Theopercula of the upper and lower gills were then removed complete with the gill arches and the opercular membrane dissected free and mounted in an Ussing chamber.
Eels were anesthetized with ethyl m-aminobenzoate methanesulfonate salt (MS-222) dissolved at 1gm/litre of salt water for 30 min. The eel was decapitated and the swim bladder removed folowing midline laparotomy and careful dissection. The swim bladder was opened along the dorsal aspect and mounted in the Ussing chamber. Skin from the eel was alsosimilarly treated. The apical side of the swim bladder membrane was the luminal surface, for the skin it was the external surface. The muscle was dissected from the skin prior to mounting in the Ussing Chamber.

The Ussing chamber was filled with modifiedForster's solution containing (in mM): $Na^+$, 40; $K^+$, 2.5; $Mg^{2+}$, 1; $Cl^-$, 47.5; HEPES, 5; and Glucose, 5 at 14°C and pH 7.5. The chamber temperature was maintained at 14°C by a circulating water pump and to reduce the unstirred layer the chambers were stirred vigorously with a magnetic stirring bar. Water and urea permeabilities were determined as described (Negrete et al. Am J Physiol 1996:271;F886894) using isotopic water and urea. In the case where oxygen permeability was to be measured the chamber lids had an Oxygen microelectrode placed through it (MI-730 Microelectrodes Bedford NH). This electrode was calibrated by passage of 95% $O_2$/5%$CO_2$ gas through the Forster's solution, then room air (21% $O_2$) and finally $N_2$ (0% $O_2$ ) through the solution. Each calibration point was made when the solution had reached a steady state reading. Then the basolateral side chamber was carefully sealed and the $O_2$ level in that chamber measured continuously by interfacing the electrode with Maclab (AD Instruments, Mountain View, CA). $O_2$ flux rates were determined by fitting the curve describing the change of $O_2$ on the apical side over time to a single exponential function. Permeability was calculated be using the surface area of exchange as previously described (Lewis SA et alMethods Enzymol 1975:192;632-650, Negrete H et al

Am J Physiol 1996:271;F886-894). Electrical measurements of transepithelial resistance, voltage and short circuit currents were made prior to placement of the $O_2$ electrodes.

The observed transepithelial resistance, and permeabilities to water, urea and oxygen are shown Table 1.

Table 1: Observed Permeabilites of water, urea and oxygen

| | TER Ohm $cm^2$ (n) | Pd(obs) Water $x10^{-6}$ cm/sec (n) | Pd(obs) Urea $x10^{-6}$ cm/sec (n) | Pd(obs) Oxygen $x10^{-5}$ cm/sec(n) |
|---|---|---|---|---|
| Flounder Operculum | 1711 ± 312 (11) | 6.6 ± 0.1 (7) | 0.51 ± 0.07 (7) | 34.5 ± 5.4 (6) |
| Eel Swim Bladder (Cephalic end) | 1925 ± 390 (12) | 5.6 ± 1.3 (12) | 0.68 ± 0.16 (7) | 12.5 ± 0.6 (5) |
| Eel Swim Bladder (Caudal End) | 1646 ± 366 (12) | 7.1 ± 2.2 (12) | 2.4 ± 1.1 (7) | - |
| Eel Skin | 2359 ± 304 (16) | 2.4 ± 0.3 (16) | 1.27 ± 0.61 (6) | 18.9 ± 2.5 (3) |

Several efforts were made to calculate the unstirred layers, including butanol fluxes, and measurements following permeabilization of the epithelia using detergents (SDS, Triton X-100) or mechanical puncture with fine needles. For the operculum, the unstirred layer thickness using butanol was 0.25 cm, a value which compares favorably with the thickness of 0.1 cm observed when mammalian urinary bladder specimens are examined in these chambers. However, butanol permeabilities of swim bladder and eel skin were extremely low, leading to calculated thickness of unstirred layers of 2.3 – 4.7 cm, which were unreasonable values. On the basis of these thicknesses, the predicted permeation of oxygen through the unstirred layer was 5 fold lower than the observed permeabilities, indicating that the butanol was not useful for measuring unstirred layers in these preparations. Unfortunately, detergents did not dissolve well in the medium, and were ineffective. However, puncturing tiny holes in the preparations led to prompt equilibration of isotopes across them, indicating that the unstirred layers likely posed a small barrier to flux when compared with the preparations themselves.

Previous measurement of oxygen permeabilities of eel swim bladder (Kutchai et al Comp. Biochem.Physiol. 1971 :39A;119-123) gave values similar to those which we obtained ranging from 4.5 to35.3 $x10^{-5}$ cm/sec with a mean of 17.6 $x10^{-5}$ cm/sec (n=5). This is within the range of our observed values.

If the unstirred layer effects were relatively minor when compared with the observed permeabilities, we can draw the following conclusions:

1. Each of the preparations exhibited relatively high resistances.
2. Each of the preparations exhibited water and urea permeabilities which were strikingly low, lower than those of mammalian bladder by a factor of 10fold in the case of water. In the case of the operculum and the eel skin, these low permeabilities likely reduce markedly

dissipation of the osmotic gradients between the interior of the eel or flounder and the ocean or fresh water outside.

3. Oxygen permeabilities were lower in swim bladder than in opercula. In swim bladder, the presence of guanine crystals is thought to cause exceptionally low oxygen permeability. The swim bladders we studied were clear and not irridescent, suggesting that they did not have very many guanine crystals. These results suggest that the lower permeability of the swim bladders with respect to operculum could be caused by differences in permeation of oxygen through the lipids of the two preparations, or possibly by the presence of specialized oxygen conduction capacity in the operculum. The similarities in water and urea permeabilities between eel swim bladder and flounder operculum suggest that their membrane lipids should have exhibited similar oxygen permeabilities, so that it is possible to speculate that opercula may have oxygen pores. This possibility will be explored in future studies using purified vesicles prepared from gill surface membranes, and stopped-flow techniques, which avoid the difficulties of unstirred layer effects.