FLUORESCENCE RATIO MEASUREMENT OF LUMINAL pH IN SINGLE PERFUSED PROXIMAL II SEGMENTS OF THE DOGFISH KIDNEY (SQUALUS ACANTHIAS).

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Elasmobranch fish elaborate an acidic urine with a pH of 5.7 and exhibit a remarkable capacity for renal proton secretion. In the little skate, Raja erinacea, the proximal II segment is an important site of proton secretion. Using pH-selective microelectrodes, Silbernagl et al. (Bull. MDIBL 26: 156, 1986) determined that the acidification process in this segment is independent of carbonic anhydrase and appears to involve basolateral Na:K:Cl symport and Cl:HCO_3 exchange. The present studies were undertaken to assess the feasibility of using the fluorochrome bis (carboxyethyl carboxyfluorescein), BCECF, for measurement of luminal acidification in the in vitro perfused proximal II segment from the kidney of another elasmobranch, Squalus acanthias. 700 micron-long segments of proximal II tubules from dogfish were perfused and bathed at 17-19°C in symmetrical shark Ringer solution. Axial changes in luminal fluorescence at 530 nm was monitored using a microcomputer-based digital image processing system. At slow perfusion rates, the reduction in the 490/450 nm fluorescence excitation ratio indicated luminal acidification. At higher rates of tubule perfusion, axial pH was clamped at the pH of the external media, 7.4. Removal of peritubular K resulted in a further reduction of luminal pH. These observations demonstrate the feasibility using BCECF to study the cellular mechanism of urinary acidification in discrete microperfused nephron segments. Our preliminary data indicate that luminal acidification obtains in the dogfish proximal II segment. The enhanced acidification following bath K deletion is consistent with the observations of Silbernagl et. al. that peritubular furosemide, an inhibitor of Na:K:Cl symport, lowered luminal pH. (These studies were supported grant DCB 87-02159 from the National Science Foundation).