Renal Bicarbonate Reabsorption in Marine Fish, represented by *Squalus acanthias*, is Based on H^+ Secretion Independent of CO_2

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Nearly fifty years ago, it was found that urinary pH in the marine dogfish and sculpin is fixed at about 5.8 (W.W. Smith, J. Cell Comp. Physiol. 14:95, 1939). No one has succeeded in bringing this value past pH 6.4, despite massive loading of $NaHCO_3$ or other buffers or metabolic inhibitors, including those of carbonic anhydrase (Hodler, et al., Am. J. Physiol. 183:155, 1955). It became clear that the excretory kidneys of *S. acanthias* and other elasmobranchs contained no carbonic anhydrase, and the same seems true, on lesser evidence, for marine teleosts (Maren, Physiol. Rev. 47:595, 1967). A small amount of the enzyme found in renal tissue of marine fish is attributable to hematopoetic tissue. It was puzzling how H^+ secretion and HCO_3^- reabsorption could occur in the absence of carbonic anhydrase. The calculated uncatalyzed rate of H^+ formation from CO_2 appeared significantly below the observed HCO_3^- reabsorptive or H^+ secretory rates, either *in vivo* (Maren, J. Pharm. Expt. Therap. 139:129, 1963) or in the isolated kidney *in situ* (Deetjen and Maren, Pflügers Arch. 346:25, 1974) of elasmobranch species.

Based on these data, I had supposed an independent mechanism for HCO_3^- reabsorption, possibly an active HCO_3^- pump, since transepithelial potentials were close to zero, and there was no evidence that HCO_3^- gradients were developed along the nephron by the reabsorption of water (Maren, 1963, 1967; Deetjen and Maren, *vide supra*).

We have found now, however (Swenson and Maren, Am. J. Physiol. 250:288, 1986) that capacity of the kidneys of *S. acanthias* to secrete acid far exceeds the uncatalyzed rate of hydration or hydroxylation of CO_2 and appears virtually unlimited. We concluded that renal acid formation in this species was dissociable from CO_2 metabolism.

Application of these H^+ secretory rates to those of renal HCO_3^- reabsorption yields a surprising result. As Table 1 shows, the normal filtered = reabsorbed HCO_3^- rate is about 25 $\mu eq \cdot hr^{-1}$ per kg (GFR = 3 $ml \cdot hr^{-1}$ per kg x plasma HCO_3^- of 8 $\mu eq/ml$), and this can be increased at least 8-fold by HCO_3^- loading (Boylan, Bull. MDIBL 13:17, 1973; Swenson and Maren, *vide supra*). Even in these extreme experiments, no HCO_3^- appears in the urine. However, renal acid secretion when stimulated by infusion of buffer approaches 400 $\mu eq \cdot hr^{-1}$ per kg (Table 1), enough to react with any conceivable amount of filtered HCO_3^-, converting this species to the readily reabsorbable $H_2CO_3 \rightleftarrows CO_2$. This rate is far in excess of the uncatalyzed hydration or hydroxylation of CO_2, which is calculated as about 30 $\mu eq \cdot hr^{-1}$ per kg for kidneys of *S. acanthias* (Swenson and Maren, *vide supra*). It must be concluded that the secretion of H^+, and hence HCO_3^- reabsorption are mediated by acid formation independent of CO_2 (Figure 1). The idea that renal HCO_3^- in the elasmobranch was reabsorbed as such, therefore, must be discarded. This was not evident until these new data were available, showing that H^+ secretion was not limited by rates or properties of CO_2 hydration.

TABLE 1

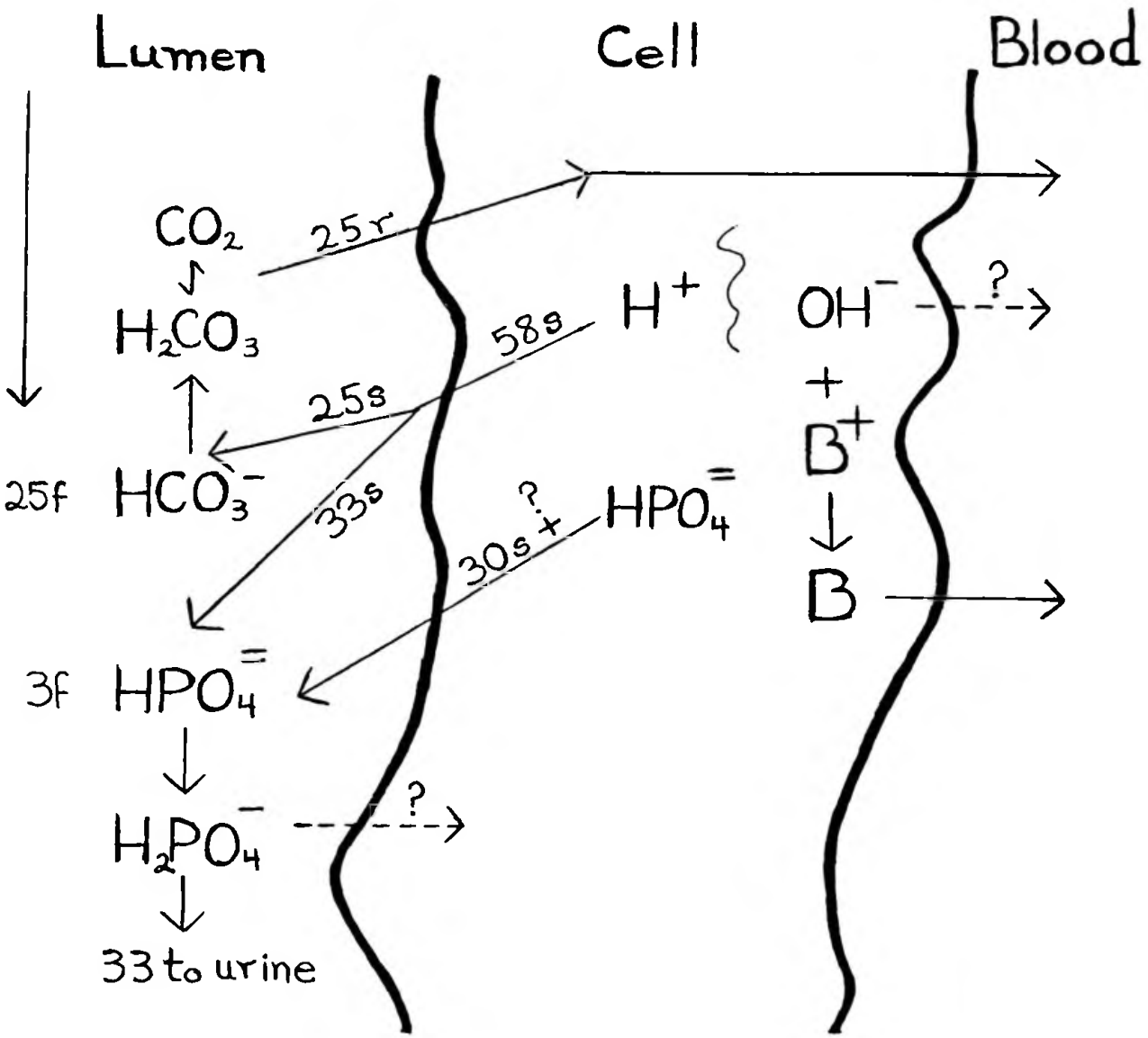
RENAL HANDLING OF H^+ AND HCO_3^- IN DOGFISH, COMPARED TO DOG

Filtered = Reabsorbed HCO_3^-	$\mu eq \cdot hr^{-1}$ per kg	
	Dogfish	Dog
Normal	25†	4000
Maximum, HCO_3^- load	>200‡	10000
Acid Output		
Normal	33	20
Buffer Stimulated	>390*	>1200

In dogfish, capacity to make acid* greatly exceeds normal HCO_3^- reabsorptive rate†, and exceeds any experimentally-delivered HCO_3^-‡. H^+ formation could subserve all HCO_3^- reabsorption; it is not necessary to postulate HCO_3^- movement as such. For these rates of H^+ secretion, all filtered HCO_3^- is destroyed.

In dogfish, HCO_3^- reabsorption and H^+ secretion appear to occur at the same site ("sinus zone" -- Proximal Tubule III, see Lacy, Anat. Embryol. 173:163, 1985) and appear to be based on a single event, H^+ secretion. Its capacity appears virtually unlimited despite absence of carbonic anhydrase. The very low (2 mm Hg) pCO_2 and low temperature nearly eliminate the uncatalyzed reactions of CO_2 chemistry (Swenson and Maren, vide supra).

Figure 1



The normal renal secretion of H^+ to reabsorb HCO_3^- and to produce acid urine in S. acanthias. Numbers are rates in $\mu eq\ hr^{-1}$ per kg fish. Subscripts f, s and r mean filtered, secreted and reabsorbed. The value of s for PO_4 is net cell → lumen; more may be secreted and some reabsorbed. The nature and site of buffering (B) of OH^- without CO_2 is not known. It may be within the cell (as shown), or by blood.

Table 1 includes comparative data from the dog, see Maren, 1967, Table 20 and Garg, J. Pharm. Expt. Therap. 194:96, 1975. It is evident that in the mammal the HCO_3^- reabsorptive rate is enormously greater than in the fish (since both filtration rate and plasma HCO_3^- are much higher). The acid output is not very different between fish and mammal. The essential difference between species is the presence of carbonic anhydrase. In the mammal, some 80% of proximal HCO_3^- reabsorption and all of (distal) H^+ formation depends on the enzyme. The contribution of the uncatalyzed reaction to either process probably is negligible. From this intra-species comparison, it seems likely that the carbonic anhydrase independent mechanism for H^+ formation in fish has not been preserved in mammals, but much more work is needed on this point.

The acidity of urine pH in marine fish is surely connected, as Homer Smith said half a century ago, to the fact that the urinary constituents of Ca^{++}, Mg^{++}, $PO_4^=$, $SO_4^=$ are relatively insoluble at pH greater than about 6.4 demanding the fixity of urine pH at 5.8. Interestingly, the rate and concentration of urinary H^+ in S. acanthias is quite constant, at about 33 $\mu eq \cdot hr^{-1}$ per kg and 33 mM (Figure 1). This is matched by phosphate, which is secreted actively. It seems reasonable to suppose that the machinery for H^+ and $PO_4^=$ secretion evolved in parallel fashion, linked closely to cellular metabolism. It is no accident that H^+ secretion cannot be influenced substantially by any known pharmacological or electrolyte manipulation; it appears tied to the life of the animal.

Finally, it should be noted that the minerals that endanger the fish at pH > 6 are not, as earlier thought, chiefly the salts of magnesium, despite their high concentration in the urine. A review of solubilities and some simple laboratory experiments show that the first substance to precipitate (at pH 6.7) is $CaHPO_4$, a compound responsible for renal stones in man. In vertebrate evolution, the necessary acquisition of pH flexibility has had its price: Loss of the renal mechanism that would prevent this type of calcification.

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