such a value can account for neither basal nor stimulated acid output. Furthermore, the failure of this reaction adds further to the concept that protolysis of water (reaction 2) must be the primary reaction in the generation of protons and not CO_2 hydration and carbonic acid formation.

Reactions 2 and 3 show acid secretion in terms of H_2O protolysis and hydroxylation of CO_2 as a means of buffering OH^-. For the latter reaction

$$V = k_2 \cdot [CO_2] \cdot [OH^-] = 29 \text{ mM/hr}$$

where $k_2 = 4000 \text{ sec}^{-1} \text{ M}^{-1}$ at 16°C, $CO_2 = 0.2 \mu M$ and OH^- is given a very high value of 10^{-5} M (pH = 9.0). This may be obtained in certain regions of the cell (i.e., the antiluminal border) but for purposes of argument we use it, realizing the average cell pH is much lower. Using the reaction volume of 2.5 ml (vide supra) then

$$V = 72 \mu \text{ moles/hr} \cdot \text{kg fish}$$

a value only 20% of the ovserved stimulated acid secretion.

Related experiments by Garvey and Maude (Am. J. Physiol. 240:F306, 19) in the isolated perfused rat kidney show a similar failure of the uncatalyzed CO_2 reactions to match acid secretory rates, when the kidney is perfused with $CO_2 HCO_3^-$ free Ringer's solutions and carbonic anhydrase is inhibited. Our experiments extend these observations in several salient ways; we studied acid secretion in an intact animal without renal carbonic anhydrase and attempted to augment the process.

The negative result with methazolamide suggests two important points. First, it rules out a small but heretofore undetectable amount of renal carbonic anhydrase whose activity might only be essential and manifested during such high rates of acid secretion. Secondly, red cell enzyme (inhibited by methazolamide) is not necessary for buffering of OH^- or other base generated by acid secretion.

Sanders et al (Am. J. Physiol., 225:1311, 1973) showed that acid secretion by the in vitro frog stomach is reduced but not abolished in the absence of exogenous CO_2. Their conditions were radically different and carbonic anhydrase was not inhibited. However, they found that the H^+ secretory rate could be returned to normal levels in CO_2 free conditions by the use of appropriate buffer concentration in the bathing media (HPO_4/H_2PO_4 = 25 mH and pH = 4.4). They attributed this to recycling of endogenous metabolic CO_2. Such an explanation could not explain the present results or conclusions, since we assume a constant concentration of CO_2 - as did Garvey and Maude (vide supra).

In conclusion, renal acid secretion appears to involve primary protolysis of H_2O, with H^+ and OH^- ion separation. This process need not be dependent upon a reaction with CO_2 or on renal or erythrocyte carbonic anhydrase. The mechanism for handling of hydroxyl ions remains unknown, but buffering by other intracellular acids (amino acids, phosphate) or direct Cl^-/OH^- exchange might be postulated. This work was supported by NIH grant HL-22258.

THE MECHANISM OF THE HYPOTONICITY-INDUCED K^+ ACCUMULATION IN SLICES OF THE RECTAL GLAND OF THE DOGFISH (SQUALUS ACANTHIAS)

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We have reported previously (Goldstein et al., Bull. MDIBL 21:3, 1981) that hypotonic (urea-free) media produced a) an efflux of urea from the slices; b) a modest increase in tissue H_2O; and c) a net influx of K^+ until the original electrochemical gradient has been reached. Several lines of evidence permitted us to exclude a direct coupling between urea efflux and K^+ influx. This report concerns the actual mechanism by which the efflux of an uncharged molecule brings about an influx of KCl into the cells.

We have first determined that it is justified to employ $^{86}Rb^+$ fluxes as a measure of E^+ fluxes: The 5 min influx of the label was taken to approximate the zero-time flux. Mean values, ± S.E.M. (5 measurements): $^{42}K^+$ influx: 2.97 ± 0.13 μmol/g tissue wet wt. (W.W.): $^{86}Rb^+$: 2.52 ± 0.07 μmol/g W.W. Within the limits of experimental error the fluxes of $^{42}K^+$ and $^{86}Rb^+$ are thus commensurate.

We measured the efflux of $^{86}Rb^+$ from slices preloaded with the label by 30 min aerobic incubation (standard saline with 1 mM $^{86}Rb^+$, 0.1 μCi/ml). The wash-out of the label from the blotted slices was followed a) in standard media; b) urea-free media. The efflux curves were resolved into two cellular components: Isotonic medium (890 mosM): $P = 0.33.e^{-7.2\,t} + 0.67.e^{-0.20\,t}$; urea-free (hypotonic) medium, 550 mosM: $P = 0.32.e^{-7.5\,t} + 0.68.e^{0.123\,t}$. The significance of the difference between the rate constants in the control and hypotonic saline for the fast efflux component is further documented in Figure 1; $p < 0.001$ (two experiments) for the slower rate constants. Hypotonicity thus decreases the efflux of both components.

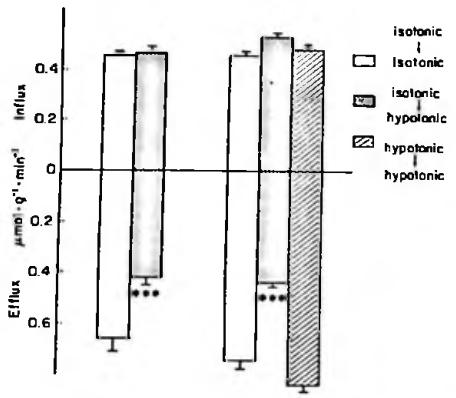

Figure 1.--Effect of saline tonicity on $^{86}Rb^+$ fluxes in slices of the dogfish rectal gland. Left pannel: Mean values, ± S.E., for 5 fish. Right pannel: 1 fish; mean values, ± S.E., of 5 measurements.

The zero-time (5 min) unidirectional fluxes of $^{86}Rb^+$ were then measured: The influx corresponds essentially to the operation of the sodium pump; the efflux is a measure of the K^+ "leak". It was assessed that under the given experimental conditions the contribution of the label in the extracellular tissue space introduced not more than a 5% error. Figure 1 (left pannel) shows that the change from isotonic to hypotonic media did not affect the $^{86}Rb^+$ influx, demonstrating that hypotonicity does not affect the Na^+-pump. On the other hand, hypotonicity significantly ($p < 0.001$) decreased the efflux of $^{86}Rb^+$ from the tissue, showing a reduction of the permeability of the K^+ channel.

The above data provide an explanation for the KCl accumulation in tissue incubated in hypotonic media: A decrease in K^+ efflux, at a constant influx, does indeed produce an increase in cell K^+.

An explanation was sought for the observation that KCl uptake by the tissue stops with the urea efflux, when also the electrochemical gradient of K^+ has reached that in the control. Therefore, zero-time unidirectional fluxes of $^{86}Rb^+$ were also measured after the tissue reached a new steady state in the hypotonic medium. Figure 1, right panel, shows that the influx was not affected during the whole procedure. On the other hand, the efflux, first reduced on transfer of the tissue from isotonic to hypotonic medium, returns at steady state to the value seen in the controls.

The data clearly demonstrate that medium hypotonicity produces a temporal decrease in the $^{86}Rb^+$ efflux, which disappears once the tissue has reached a new steady state of H_2O and electrolytes. Obviously, the cell volume control mechanism is not triggered by the actual cell volume, but by the changes in the osmotic or electrochemical gradients across the cell membrane. This study was supported in part by NIH Grant AM 12619, and by the Whitehall Foundation.

SUGAR TRANSPORT BY THE INTESTINAL MUCOSA OF THE WINTER FLOUNDER (Pseudopleuronectes americanus)

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Studies on the intestinal transport of sugars by the intestine of the flounder have been continued.

1.--GLUCOSE TRANSPORT--Previous reports have suggested that the conventional glucose-sodium cotransport is operative in the intestinal epithelial cells of the flounder (Pseudopleuronectes americanus) in spite of the fact that no net glucose transport or glucose stimulation of the short-circuit current (I_{sc}) had been found. Based on a comparison