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Evidence for a Disequilibrium pH in the Proximal Tubule of Rat Kidney.* (28079)

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Reabsorption of filtered HCO_3^- by the kidney is thought to be mediated by secretion of cellular H^+(1,2), resulting in the following intratubular reaction:

$$\underset{\text{(secreted)}}{H^+} + \underset{\text{(filtered)}}{HCO_3^-} \rightleftarrows H_2CO_3 \rightarrow CO_2 + H_2O \quad \downarrow\uparrow \quad CO_2\,\text{(plasma)}$$

Since dehydration of H_2CO_3 is not instantaneous, excess H_2CO_3 would accumulate in tubular fluid and would not be in equilibrium with plasma CO_2. Consequently, intratubular pH would be lower than that calculated by the Henderson-Hasselbalch equation using plasma pCO_2 and luminal concentration of HCO_3^-(3).

An opposing view postulates that HCO_3^- ions *per se* are reabsorbed directly(4). Were this the mechanism involved, excess H_2CO_3 would not be generated, H_2CO_3 and CO_2 would be in continuous equilibrium, and the intratubular pH would be equal to that predicted by the Henderson-Hasselbalch equation.

In the present experiments the *in vivo* intratubular pH of single proximal tubules perfused with a solution containing 60 mM $NaHCO_3$ was 6.3, or 1.5 pH units below the calculated equilibrium pH of 7.8. These results afford the first direct evidence that HCO_3^- reabsorption in the proximal tubule is mediated *via* H^+ secretion.

Methods. Sprague-Dawley rats were anesthetized and prepared for micropuncture as previously described(5). The exposed surface of the kidney was covered with Ringer's bicarbonate solution continuously equilibrated with 5% CO_2-95% O_2.

Single proximal tubules were perfused by first injecting a drop of silicone oil into the lumen through a micropipette. The oil drop was then split by injecting with a second micropipette a solution containing 90 mM NaCl and 60 mM $NaHCO_3$. The pH of this solution when equilibrated with 5% CO_2 was 7.8.

The *in vivo* intratubular pH was estimated by adding various acid-base indicators to the perfusion fluid in a concentration of 100 mg %. The color changes in the column of injected fluid were observed by direct microscopic visualization. For each indicator 10 to 15 tubules were injected and observed. In a second group of experiments carbonic anhydrase, as well as the acid-base indicators, was added to the perfusion fluid in a concentration of 100 mg %. To evaluate the ability to detect color changes in fluid columns of tubular dimensions (approximately 30 μ in diameter) samples of test solutions with the various acid base indicators were titrated to several different pH values between pH 5

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TABLE I. Estimation of Steady State Intraluminal pH of Proximal Tubules Injected with Solutions Containing $NaHCO_3$ and Acid-Base Indicators.

Acid-base indicator	pH range	Color range			Intratubular color			
					Control*		Carbonic anhydrase†	
		Acidic		Basic	Color	pH	Color	pH
Brom cresol green	3.8-5.4	Yellow	—	Blue	Blue	>5.4		
Methyl red	4.8-6.0	Red	—	Yellow	Colorless	>6.0		
Chlorphenol red	5.0-6.6	Yellow	—	Red	Slight pink	<6.6		
Brom cresol purple	5.2-6.8	"	Red Purple	Blue Purple	Red Purple	<6.8		
Brom thymol blue	6.0-7.5	"	—	Blue	Colorless	<7.0		
Phenol red	6.8-8.4	"	—	Red	"	<7.2	Red	>7.5
Cresol "	7.2-8.8	Amber	—	"	"	<7.2	"	>7.6
Meta cresol purple	7.5-9.0	Yellow	—	Purple	"	<7.5	Red Purple	>7.7
Thymol blue	8.0-9.6	"	—	Blue	"	<8.5	Colorless	<8.5
Estimated steady state pH						~6.3		~7.7

* Test solutions contained 90 mM NaCl, 60 mM $NaHCO_3$ and 0.1% indicator.
† 100 mg% carbonic anhydrase added to control test solutions.

and 9, and were then placed in capillary tubes (60 μ in diameter) for observation under the microscope. Even when viewed against a faint reddish background (to simulate the background color of the kidney) the predicted colors were easily observed.

Blood pH was measured anaerobically at 37°C with a Beckman glass electrode and a Vibron pH meter. Plasma CO_2 contents were determined by the method of Van Slyke and Neill(6).

Results. The blood pH of these rats was 7.37 ± .05 (S.D.), the CO_2 content was 23.1 ± 2.2 (S.D.) meq/l, and the calculated plasma pCO_2 was 38 ± 5 (S.D.) mm Hg.

The acid-base indicators with their pH range and color changes are listed in Table I. Perfusion fluids containing thymol blue, meta-cresol purple, cresol red, phenol red and brom thymol blue changed from deeply colored to colorless (the yellow color could not be detected against the background of the kidney) within 5 to 10 seconds after injection into the tubule, indicating an intratubular pH below 7.0. The blue color with brom cresol green and lack of color with methyl red placed the pH above 6.0, while the red-purple color with brom cresol purple and pink color with chlorphenol red indicated a pH of approximately 6.3. Thus, the intratubular pH was approximately 1.5 units below the equilibrium pH of 7.8.

Addition of carbonic anhydrase to the perfusion fluid resulted in a much higher intratubular pH. The red color with phenol red and cresol red, the red-purple color with meta cresol purple, and the lack of color with thymol blue indicated an intratubular pH of 7.7 or approximately equal to the equilibrium value.

Discussion. The *in vivo* intratubular pH of proximal convoluted tubules perfused with a solution containing 60 mM $NaHCO_3$ was approximately 6.3, a value 1.5 units below that calculated by the Henderson-Hasselbalch equation assuming equilibrium between intratubular H_2CO_3 and plasma CO_2. The difference between the steady state intratubular pH and the equilibrium pH was obliterated by adding carbonic anhydrase to the perfusion fluid, thus proving that the pH of 6.3 was the consequence of excess H_2CO_3 in tubular fluid. The concentration of excess H_2CO_3, calculated from the Henderson-Hasselbalch equation using the pH of 6.3 and the HCO_3^- concentration of 60 meq/l, was approximately 40-fold greater than the concentration of H_2CO_3 in plasma. This concentration of H_2CO_3 far exceeded the value that could be obtained by simple concentration of filtered H_2CO_3 by water reabsorption and indicated that the excess H_2CO_3 must have been generated within the tubular lumen.

These results permit the following conclusions: First, the primary event in the reabsorption of filtered HCO_3^- is not direct reab-

sorption of HCO_3^- ions *per se,* but rather is the secretion of H^+ ions which react with filtered HCO_3^- to generate H_2CO_3. Second, the accumulation of excess H_2CO_3 in tubular fluid disproves the previously suggested hypothesis(3,7) that endogenous carbonic anhydrase might be located in the luminal membrane of renal tubular cells, thereby exerting a catalytic action on dehydration of excess H_2CO_3 in tubular fluid.

Finally, the calculated pH gradient (pH_B-pH_{TF}) between blood and tubular fluid was 1.07. For a pH gradient of this magnitude to result from passive distribution of H^+ ions along an electrochemical gradient(8,9), it can be calculated from the Nernst equation [$E_T = -61.5\ (pH_B\text{-}pH_{TF})$] that a transtubular potential difference (E_T) greater than 66 mV, lumen negative to blood, would be required. Previous measurement of proximal E_T under conditions of $NaHCO_3$ diuresis gave an average value of only −20 mV(10). Therefore, it is concluded that H^+ ions are secreted into the lumen against an electrochemical gradient by an active transport process.

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Purification and Characterization of Chick Embryo Interferon. (28080)

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Various virus-host animal or virus-cell culture systems have been shown to elaborate a "soluble" substance called interferon(1), which interferes with the propagation of viruses in related host or host cell systems. Interferon appears to be an active principle in the virus interference phenomenon. Interferon, as first described by Isaacs and Lindenmann(1), was prepared in fragments of chick chorioallantoic membrane incubated with heat-inactivated influenza virus. Wagner(2) demonstrated the presence of an interferon in the allantoic fluid of embryonated eggs infected with influenza virus and other workers have demonstrated interferons in other systems. Several groups of workers (2-6) have described the biological and biophysical properties of crude or semi-purified interferons. The present report describes the biological and biophysical qualities of a highly purified interferon derived from the allantoic fluid of embryonated hens' eggs infected with the WS strain of influenza virus.

Materials and methods. Preparation of crude interferon. Nine- or 10-day-old embryonated eggs were inoculated by the allantoic route with $10^{4.5}$ ID_{50} of WS influenza strain RIE 5372 virus obtained from Dr. I. Tamm. The eggs were incubated at 36°C for 84-96 hours after which they were chilled at 4°C and the allantoic fluid containing the interferon was harvested. The crude fluids were centrifuged in the cold for 20 minutes at 1500 rpm and the supernates were stored at 4°C for periods up to 14 days prior to processing for purification. In vitro *assay for interferon activity.* Serial 2-fold dilutions of the interferon sample were diluted in mixture 199 containing 1% calf serum and antibiotics, and one ml amounts were added in