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Research Article

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Proximal Bicarbonate Reabsorption during Ringer and Albumin Infusions in the Rat

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Abstract Several studies have clearly shown that extracellular volume expansion is associated with suppression of whole kidney bicarbonate reabsorption, although little is known concerning the single nephron correlates of this response. More recently, attention has also been focussed on bicarbonate transport in attempts to identify a possible role for this ion in enhancing the rate of net fluid efflux by proximal tubules. To further explore proximal tubular bicarbonate handling in the rat, we carried out recollection micropuncture studies to assess the effects of infusions of modified Ringer or salt-poor hyperoncotic human albumin. With stable levels of arterial P_{CO_2}, plasma [HCO_3^-] or plasma [K^+], marked suppression of fractional HCO_3^- reabsorption occurred: during Ringer infusion fractional reabsorption fell by 31% ($P < 0.001$) while during albumin infusion a decrease of 20% ($P < 0.001$) was observed. Despite this, absolute net HCO_3^- reabsorptive rates did not change significantly. Simple and partial correlation analysis of single tubular responses revealed strong linkage effects between changes in absolute net reabsorptive rates for HCO_3^- and H_2O in both types of infusion; the partial r was 0.91 ($P < 0.001$) and 0.94 ($P < 0.001$) during Ringer and albumin infusions, respectively.

We conclude that under these free-flow conditions, Ringer and albumin infusions do not suppress absolute net HCO_3^- reabsorption by proximal tubules, and that strongly linked changes in absolute HCO_3^- and H_2O fluxes are characteristic of both protocols.

INTRODUCTION

In the rat (1), dog (2), and man (3), recent studies have demonstrated that whole kidney bicarbonate reabsorption is decreased in association with extracellular fluid (ECF)[1] volume expansion. However, the single nephron correlates of this response are not yet completely defined. Although it is generally assumed that ECF volume expansion decreases bicarbonate reabsorption by the proximal convoluted tubule (1, 3, 4), this view is only supported by a preliminary report (5) in which fractional bicarbonate reabsorption was estimated from measurements of tubular fluid chloride concentrations and fractional water reabsorption. While there is no a priori reason to doubt the inference that fractional bicarbonate reabsorption by proximal tubules is suppressed concomitantly with ECF volume expansion, it is possible that if filtered bicarbonate loads increase (as in the whole kidney studies noted above), absolute net bicarbonate reabsorption could remain unchanged. In fact, in the case of net fluid efflux, it has already been clearly demonstrated that fractional measurements are an inadequate guide to absolute reabsorptive rates (6, 7). Direct measurements of absolute net reabsorptive rates are important not only to an assessment of distal delivery rates of bicarbonate during volume expansion, but also to an understanding of in vivo free-flow transport characteristics of proximal tubular epithelium.

Measurements of absolute proximal bicarbonate reabsorption are also relevant to the conflicting evidence concerning the influence of bicarbonate in facilitating proximal sodium and water efflux (8–10). It is obvious, of course, that free-flow measurements in vivo can provide only limited insight into tubular transport mechanisms, and do not distinguish between reabsorptive characteristics of different portions of the proximal tubule. Nevertheless, we believe that evaluation of linkage effects between water and bicarbonate movements pro-

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[1] *Abbreviations used in this paper:* ANOVA, analysis of variance; BW, body weight; ECF, extracellular fluid.

vide a necessary step in discerning any relevance for net fluid retrieval by single nephrons of transport schemes which attribute a central role to bicarbonate ion (11, 12). Indeed, we have already attempted to assess bicarbonate and water movements in several situations (13–15) but in each of our previous preparations it was possible that the induced changes in acid-base and potassium balance had a more direct influence on fluxes of bicarbonate than of water. On the other hand, infusions of Ringer or albumin are associated with several factors thought to diminish net proximal fluid reabsorption. Such factors as altered peritubular oncotic pressure (16), and changes in renal perfusion pressure (17) have not been considered to directly influence bicarbonate movements. Accordingly, it appeared to us that infusions of Ringer or hyperoncotic albumin would provide a uniquely different framework in which to assess free-flow HCO_3 and H_2O linkage effects.

With these considerations in mind we carried out studies whose specific objectives were (*a*) to examine free-flow proximal bicarbonate reabsorption during infusions of modified Ringer and hyperoncotic albumin solutions, and (*b*) to determine in each situation to what degree absolute net bicarbonate and water movements are linked.

In our experiments although fractional bicarbonate reabsorption fell markedly in both protocols, we were unable to demonstrate a decrease in absolute net bicarbonate reabsorption. Tight coupling of bicarbonate and water movements was characteristic of both preparations.

METHODS

Recollection micropuncture experiments were carried out on male Sprague-Dawley rats weighing 250–325 g. All animals had free access to a stock diet and tap water before the induction of anesthesia with Inactin, 130 mg/kg body weight (BW). Polyethylene catheters were inserted into the left carotid artery and right jugular vein for continuous blood pressure monitoring, blood sampling, and infusions. The left ureter was catheterized with PE50 polyethylene tubing. The temperature of animal and kidney surface was kept constant at approximately 38°C by servo-regulated devices using thermistors within the rectum and in the oil layer on the kidney surface. No animals were studied unless blood pressure was greater than 100 mm Hg and transit time was less than 13 s. Other details of the micropuncture preparation and calculations have already been described (13).

Ringer infusion experiments. 10 of these experiments were carried out. In the hydropenia period the animals received 0.5% BW/h of a modified Ringer solution ($Na = 146$ meq/l, $Cl = 124$ meq/l, $HCO_3^- = 26$ meq/l, $K+ = 4$ meq/l) after an inulin (inulin-methoxy-H^3, New England Nuclear Co., Canada) prime in a volume of 0.25 ml. After approximately 45 min, an infusion equal to 10% BW of the same solution was administered during the next hour. Thereafter, during the recollection period a sustaining infusion of 5% BW/h was maintained. Extensive preliminary experiments indicated that lesser sustaining infusions were associated with progressive increases in hematocrit and plasma protein concentrations, occasionally even to normal levels. Usually five late proximal segments were identified and mapped approximately 30 min before the first puncture. Successful recollections were obtained on two to five of these tubules. In addition, during the expansion period "fresh" or nonrecollected tubules were also punctured for the purpose of comparing single nephron glomerular filtration rate (see Results).

Albumin infusion experiments. 10 experiments were carried out in which a greater degree of hydropenia was induced by infusion of the modified Ringer solution at a rate of 0.3% BW/h. Thereafter, during a period of 30 min 25% salt-poor human albumin was infused at a rate equal to 2% of the BW, and sustained during the recollection period at the rate of 0.5 BW/h. These infusion rates were chosen to achieve a broad range of vascular volume ex-

TABLE I

*Various Effects of Modified Ringer Infusion (10 Rats) and Hyperoncotic Albumin Infusion (10 Rats)**

	Modified ringer infusion		Albumin infusion	
	Hydropenia	Infusion	Hydropenia	Infusion
BP, *mm Hg*	117±2	108±2‡	126±4	117±3‡
Hematocrit, %	48.26±0.49	40.71±0.76‡	51.80±0.69	34.04±0.52‡
Plasma Protein, *gram/100 g*	5.07±0.11	3.54±0.11‡	5.12±0.07	7.07±0.10‡
Plasma Na^+, *meq/liter*	144.6±1.2	143.7±1.4	142.8±0.8	145.0±0.8§
Plasma K^+, *meq/liter*	4.17±0.12	3.93±0.15	4.59±0.09	4.08±0.10‡
Plasma Cl^-, *meq/liter*	102.0±1.0	107.1±1.6‡	97.11±1.34	94.67±1.27
Plasma HCO_3^-, *meq/liter*	26.86±0.28	26.50±0.39	28.70±0.43	30.16±0.48‡
Arterial P_{CO_2}, *mm Hg*	43.31±0.94	39.52±0.92‡	47.25±0.92	48.44±1.03
Kidney GFR,‖ *ml/min*	1.10±0.11	1.46±0.14‡	1.13±0.05	1.21±0.06
U/P Inulin‖	778±104	88±33‡	1019±122	76±26‡
Na Excretion, *μeq/min*	0.11±0.02	8.58±2.02‡	0.13±0.03	2.49±0.47‡

* Values expressed as mean±SEM.

‡ Significant at 0.01 level or less.

§ $P < 0.05$.

‖ GFR, glomerular filtration rate; U/P, urine/plasma.

TABLE II

Recollection Micropuncture Data for Modified Ringer Infusion (10 rats, 32 tubules) and Hyperoncotic Albumin Infusion (10 rats, 34 tubules)

	SNGFR		TF/P_{In}		TF/P_{HCO_3}		Fract. HCO_3		Fract. H_2O		Abs. H_2O Reab.		Abs. HCO_3 Reab.	
	H*	E	H	E	H	E	H	E	H	E	H	E	H	E
	nl/min										*nl/min*		*peq/min*	
Modified ringer infusion														
Mean	31.68	47.91	2.00	1.30	0.40	0.68	0.79	0.48	0.48	0.22	15.20	11.43	725.15	656.56
±SEM	1.70	2.68	0.06	0.02	0.02	0.03	0.02	0.03	0.02	0.02	0.92	1.12	37.56	57.16
Sensitivity	5.99		0.11		0.05		0.05		0.03		2.92		136.00	
Significance	$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$		$P > 0.05$		$P > 0.05$	
Albumin infusion														
Mean	39.63	47.92	2.34	1.67	0.30	0.55	0.86	0.66	0.55	0.39	21.57	18.93	1061.82	1027.61
±SEM	1.58	2.08	0.09	0.04	0.02	0.02	0.01	0.02	0.018	0.014	0.814	1.157	47.50	65.09
Sensitivity	4.57		0.15		0.03		0.03		0.03		2.48		122.34	
Significance	$P < 0.01$		$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.05$		$P > 0.05$	

Sensitivity refers to the absolute difference in mean, if present, that could be detected as significant at the 0.05 level of confidence.

* *Abbreviations used:* Abs H_2O reab, absolute reabsorptive rate of H_2O; Abs HCO_3 reab, absolute reabsorptive rate of HCO_3; E, expansion; Fract HCO_3, fractional HCO_3 reabsorption; H, hydropenia; SNGFR, single nephron glomerular filtration rate; TF/P_{HCO_3}, tubular fluid to plasma bicarbonate concentration; TF/P_{In}, tubular fluid to plasma inulin concentration.

pansion and to ensure that in both initial and recollection micropuncture periods hematocrit and plasma protein concentrations were constant. "Fresh" tubules were punctured in this protocol as well.

Analytical procedures. Tritiated inulin was counted in an Aquasol-water gel in an ISOCAP 300 scintillation counter (Searle Analytic Inc., Des Plaines, Ill.) with sample activity three to eight times background. Plasma protein was measured using a hand refractometer, chloride using a Radiometer titrator, and sodium and potassium using the IL343 flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.). Duplicate measurement on 10-μl samples of plasma or urine permitted two or three determinations in each micropuncture period. Acid-base measurements on blood have been detailed previously (13).

Tubular fluid samples were analyzed for bicarbonate in vitro using the micro glass pH electrode previously described (18). In these studies we have assumed that the arterial P_{CO_2} is identical with the peritubular capillary P_{CO_2} and that there is neither a disequilibrium pH or disequilibrium P_{CO_2} in the *in situ* tubular fluid (18). In the 20 experiments reported here mean P_{CO_2} drift of the chamber was 1.0±0.2 mm Hg and mean mV drift was 1.4±0.3 mV.

Statistical methods. Where appropriate (Table I) significance testing was carried out using the paired *t* test.

TABLE III

*Correlation Analysis of Differences between Absolute Net Water and Bicarbonate Reabsorptive Rates in Various Experimental Protocols**

Protocol	Simple correlation	Partial correlation	Reference
Ringer Infusion	0.903	0.909	Present data
	$P < 0.001$	$P < 0.001$	
Albumin Infusion	0.929	0.942	Present data
	$P < 0.001$	$P < 0.001$	
K loading normal rats	0.901	0.937	Levine et al. (15)
	$P < 0.001$	$P < 0.001$	
K loading K-depleted rats	0.937	0.957	Levine et al. (15)
	$P < 0.001$	$P < 0.001$	
Correction of NH_4Cl acidotic rats	0.698	0.636	Levine and Nash (14)
	$P < 0.001$	$P < 0.05$	
Sham correction of NH_4Cl acidotic rats	0.943	0.950	Levine and Nash (14)
	$P < 0.001$	$P < 0.001$	
Acute hypercapnia in Ringer-infused rats	0.834	0.711	Levine (13)
	$P < 0.001$	$P < 0.01$	
Acute hypercapnia in bicarbonate-loaded rats	0.858	0.952	Levine (13)
	$P < 0.001$	$P < 0.001$	

* See also text.

In the case of comparison of "fresh" versus recollected tubules, analysis was done for each animal using the unpaired t test. In addition an over-all comparison was made on group data using unpaired or paired t testing. All recollection micropuncture data (e.g. Table II) were subjected to rigorous, paired analyses of variance techniques, which account for differing numbers of pairs in each experiment as well as animal-treatment interaction effects (see Appendix). Sensitivity testing was carried out as previously described (15). Finally, correlation analyses relating to linkage effects (Table III) were done using simple and partial correlation techniques following standard methods. Values are expressed as mean±SEM.

RESULTS

Figs. 1 and 2 and Table I summarize data characterizing the whole kidney and other responses to the two kinds of infusions. In both protocols hematocrit and plasma protein concentrations were stable during the expansion period as were P_{CO_2}, plasma [HCO_3^-], and plasma [K^+]. However, as noted in Table I several parameters showed minor but statistically significant changes if comparisons are made between the hydropenia and the expansion periods. Of these only plasma chloride concentrations seem to have changed substantially presumably related to the concentration of anionic albumin binding sites. The tendency for higher values for urine/plasma inulin ratios, hematocrit, and plasma protein concentration, in the hydropenia period of the albumin protocol appears to reflect the lower infusion rate (see Methods) used in this period when compared to the modified Ringer-infused animals.

Table II summarizes the micropuncture data, and for each parameter, in addition to significance values, the sensitivity of the measurements are offered. This value refers to the change detectable at the 5% level of confidence if such a difference was present. Both fractional

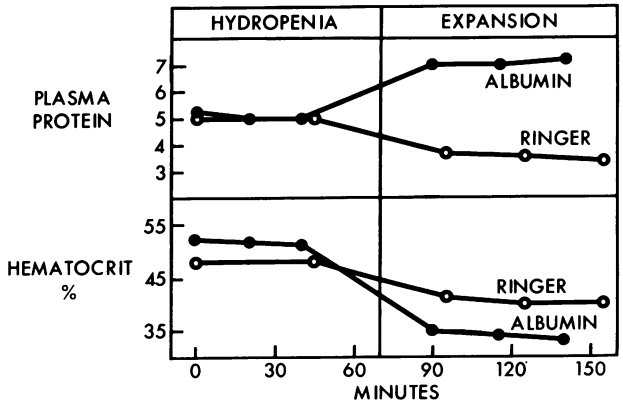

FIGURE 1 Hematocrit and plasma proteins. Effects on hematocrit and plasma protein concentration of modified Ringer and hyperoncotic albumin infusions. Symbol ○ = modified Ringer infusion; ● = albumin infusion. Plasma protein = gram per 100 g. See also text.

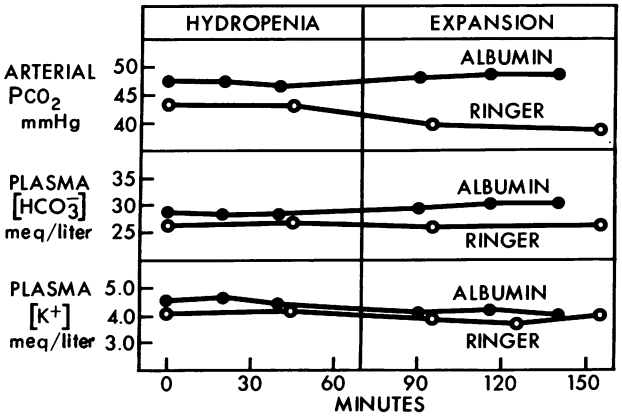

FIGURE 2 Various plasma parameters. Effects of modified Ringer and hyperoncotic albumin infusions on arterial P_{CO_2}, plasma bicarbonate concentration, and plasma potassium concentration. Symbol ○ = modified Ringer infusion, ● = albumin infusion. See also text.

water and fractional bicarbonate reabsorptive rates fell markedly in each protocol, yet in neither, were we able to show a significant fall in absolute net bicarbonate reabsorption. It is of great interest (see Discussion and Appendix) that absolute net water reabsorption during Ringer infusion was not significantly decreased by the present analysis of variance techniques despite the apparent difference in mean values in relation to their standard errors.

Table III and Fig. 3 show data related to linkage effects between absolute reabsorptive rates for bicarbonate and water. Fig. 3 shows that when the changes in absolute net reabsorptive rates for water are plotted against the change in absolute net reabsorptive rates for bicarbonate (value for hydropenia minus value for expansion i.e. $\Delta ABS.H_2O$ vs. $\Delta ABS.HCO_3$) for each tubule, a linear distribution is evident. The slope of the lines for the two kinds of expansion are not significantly different from each other. Most important is the very high value ($P < 0.001$) for both the simple and partial correlation coefficients (Table III), thus indicating a strong degree of linkage between absolute net water and bicarbonate movements. Table III also summarizes correlation data on previously published (13–15) recollection micropuncture experiments in which absolute net bicarbonate and water fluxes were also measured but not analyzed in this manner.

Recollection vs. Fresh Tubules. In 12 of the 20 experiments during expansion period fresh tubules ($n = 28$) were punctured and compared with the recollection values ($n = 42$) using the unpaired t test for each experiment. There was no statistically significant result ($P > 0.05$) in all but one case. Further, the pooled analysis using the unpaired t test on group data and the paired t test using the mean of several tubules for each

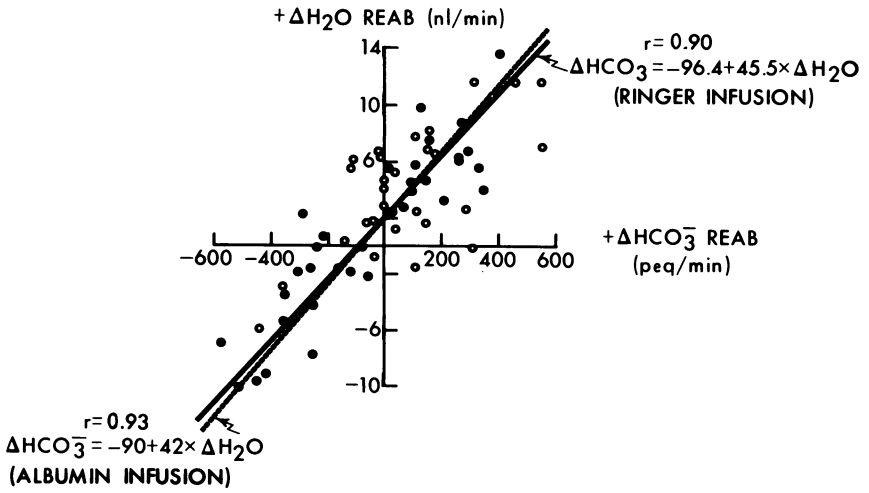

FIGURE 3 Relation of differences between bicarbonate and water fluxes. Scatter diagram of the difference between absolute net reabsorptive rates of rat proximal tubules for bicarbonate and water during modified Ringer and hyperoncotic albumin infusions. The slopes of the two lines are not significantly different ($P > 0.05$). ○ = modified Ringer, ● = albumin infusion. See also text. (The following points were not plotted but were used in calculations: −973, −13.70; −1,018, −25.67; +1,250, +30.08; +889.53, +18.76; +708.54, +13.74; +647.16, +16.88).

experiment revealed no significant difference in each case. The mean±SEM were (*a*) unpaired: 48.03±1.9, 47.77±2.2 nl/min, (*b*) paired: 47.27±2.54, 46.72±2.89 nl/min, for recollected and fresh tubules, respectively.

DISCUSSION

Our experiments show that although Ringer and albumin infusions markedly suppress proximal fractional bicarbonate reabsorption, absolute net bicarbonate reabsorption is not significantly decreased. In addition, we have demonstrated that in both protocols a highly significant degree of linkage exists between absolute net water and bicarbonate fluxes.

It is interesting to consider possible mechanisms underlying the observed changes in water and bicarbonate movements. Factors reported to influence proximal water reabsorption include blood pressure (19), renal cortical compliance (20), hydrostatic pressure or renal plasma flow (17), angiotensin II (21), peritubular oncotic pressure (16), and the recollection technique itself (7). Particularly noteworthy are dog studies by Ott et al. (22) who evaluated the role of peritubular oncotic pressure during ECF volume expansion and of Knox et al. (23) who demonstrated that in the absence of increases in parathyroid hormone concentration, preferential expansion of plasma volume had no effect on proximal water reabsorption. Concerning these possible influences, we are only able to assert that in our hands blood pressure fell in every animal studied, so that no blood pressure induced proximal tubular suppression of net fluid efflux is expected. Similarly, we are able to exclude the possibility that filtered loads were artifactually raised by the technique of recollection. The other factors listed (as well as several not noted) represent possible concomitants of Ringer and albumin infusions whose influence on water reabsorption we have not isolated.

We attempted to prevent changes in plasma bicarbonate concentration, arterial P_{CO_2}, and plasma potassium concentration since each of these factors may alter bicarbonate fluxes (13–15). Our results indicate these parameters changed minimally or not at all.[2] However, in the dog, Crumb et al. (24) reported that increases in plasma calcium concentration enhance whole kidney bicarbonate reabsorption, independent of an inhibitory action of parathyroid extract. Accordingly, we can provide no information regarding the degree to which these factors and other possible tertiary modulators of bicarbonate reabsorption (i.e. the influence of plasma Mg or 25-hydroxycholecalciferol on parathyroid hormone activity [25, 26]) may have influenced our preparations.

The first of the objectives of this study was to examine the response of absolute reabsorptive rates for bicarbonate during Ringer and albumin infusions. As already noted, the report of Kunau et al. (5) suggested

[2] Table I shows a significant fall in P_{CO_2} in the modified Ringer protocol whereas during albumin expansion both plasma K+ and plasma HCO_3^- changed significantly. These significance values, predicated of course on the very small SEM's, have questionable biological importance. For example, we have already shown that an 80 mm Hg increase in P_{CO_2} is without effect (13).

that fractional bicarbonate reabsorption is decreased during ECF volume expansion. Clearly, our experiments support this conclusion, however, our data also show that changes in absolute reabsorptive rates for bicarbonate need not occur. Accordingly, this observation does not support the inference that reabsorptive rates for bicarbonate or hydrogen ion secretion are suppressed during both kinds of infusions. We note, however, that because of the variability in experimental designs directed to the assessment of volume expansion and nephron function (7), as well as the possible effects of other concomitants noted above, it is possible that other expansion protocols might be associated with different absolute fluxes.

The significance of the linkage effects in water and bicarbonate reabsorption must be evaluated in the context of the very fundamental differences between the two protocols we have employed. The infusions of hyperoncotic human albumin caused plasma volume to increase by more than 100% and plasma protein concentration by 38%. In rats receiving the saline-bicarbonate solution plasma volume increased by only 35% while plasma protein concentration fell by 43%. Another of the many differences was the induction of a greater degree of hydropenia in the control period of the albumin experiments in a successful attempt to unmask any tendency for absolute water reabsorption to change. These considerations appear to preclude any basis for comparing the unique effects of a change in plasma protein concentration during the two kinds of infusion. Indeed, it is because of the disparate nature of the two protocols we believe that our findings of extremely close coupling between water and bicarbonate movements as well as the virtual identity of the slopes of the regression lines (see Fig. 3) are of special interest: it would seem to be highly unlikely that driving forces and permeability characteristics for HCO_3, $H+$, or $Na+$ were comparable in both sets of experiments. For example, using tubular fluid bicarbonate concentrations to estimate TF/P_{Cl^-} ratios, it can be calculated that the chloride diffusion gradient was probably more favorable to inducing solvent drag effects in the albumin protocol than during Ringer infusion. This could be taken to imply that various changes induced by the two procedures may have a minor net effect on transport parameters for H_2O and HCO_3^-. In addition, there could be coupling at cytoplasmic transport sites as discussed by Martin (12). We recognize of course, that our data cannot provide insight into the mechanism(s) underlying the linkage demonstrated.

Several important statistical considerations are worthy of close scrutiny. As noted in the Appendix, the analysis of variance (ANOVA) technique used here differs in principle from those which we have previously presented. The model outlined is the appropriate one for analyzing micropuncture data on paired experimental protocols. In practice a statistical significance obtained by paired t testing may be reversed by using this approach due to the inclusion of an additional term representing the interaction between the animal and treatment effects. For example, in the case of absolute net water reabsorption in the Ringer protocol (Table II), despite the apparent separation of the mean values in relation to their standard error and the sensitivity estimate (which was statistically significant by paired t testing and by our previous ANOVA technique), we now obtain a null effect. Insofar as our previous ANOVA technique did not account for tubule-treatment interaction the sensitivity value in this instance is only meaningful if this term is ignored. In all other entries in Table II since results of the two ANOVA models agree, the sensitivity estimates may be accepted.

In analyzing *linkage* effects the most appropriate statistical method is correlation analysis. However, our previous discussions on linkage did not take this into consideration (14, 15). For the present data we have calculated, in addition to simple correlation (Pearsons product-moment correlation), the partial correlation between ΔH_2O and ΔHCO_3 after adjusting for animal effects. Table III indicates that both the simple and partial correlations are very highly significant in the present experiments. We have treated other paired data from our laboratory in a similar manner (Table III). With the exception of the results from acidotic rats (14) (chronically ingesting NH_4Cl and then acutely corrected with sodium bicarbonate infusions), all linkage correlations are highly significant. It may be noted that the data from acidotic rats noted above was the basis of our previous assertion that rigid coupling did not exist. Although Table III shows that even in these animals a linkage effect is present, when the partial correlation is calculated it is only of borderline significance. This and other statistical issues will be more completely discussed elsewhere.[3]

It must be strongly emphasized that the high correlations noted above between ΔH_2O and ΔHCO_3 cannot be construed as implying a causal association. Nevertheless, we are persuaded the consistency of linkage effects found in the present and related data from this laboratory contribute importantly towards an evaluation of hypotheses which attribute a central role to bicarbonate ion in the control of proximal tubular fluid efflux.

APPENDIX

The statistical analysis is a modification of our linear model (model II, paired analysis) outlined previously (15).

[3] Raman, S., and D. Z. Levine. Manuscript in preparation.

TABLE IV
ANOVA

Source of variation	Degree of freedom	Sum of squares	Mean squares	F ratio
Between animals	$n-1$	E_{yy}	$B=E_{yy}/(n-1)$	B/F
Treatments	$t-1$	T_{yy}	$A=T_{yy}/(t-1)$	A/F
Between tubules	$n^1=\sum_{i=1}^{n}(n_i-1)$	J_{yy}	$D=J_{yy}/n^1$	
Animals × treatments	$(n-1)\cdot(t-1)$	K_{yy}	$F=K_{yy}/(n-1)\cdot(t-1)$	
Error	n^{11} (by subtraction)	I_{yy} (by subtraction)	$C=I_{yy}/n^{11}$	
Total	$N-1$	$\Sigma y^2-C\cdot F$		

It may be noted that Table IV is similar to our previous ANOVA table (15) except that the error term therein has been split into the two orthogonal components viz, the animals = treatment interaction and the residual error term. Consequently, the testing for statistical significance is now undertaken using the interaction in accordance with the underlying theory.

Finally, it may be stated that the analysis (15) was a generalization of the unpaired and paired t test to cases involving sampling of unequal number of tubules per animal. The current model, however, is conceptually different from the paired t test. Consequently, the interpretation of any discrepancies in statistical significance arising in either situations should be made with caution based on the underlying difference in the mathematical model.

The improvement consists in including a term for animal-treatment interaction. A statistically significant presence of this term implies that the animals react differently to the different treatments. In other words, it arises from the 'nonadditivity' of the treatment and animal effects.

The model now reads: $Y_{ijk}=\mu+\alpha_i+\tau_j+\theta_{ij}+\gamma_{ik}+\epsilon_{ijk}$; $k=1,\ldots n_j$; $j=1,\ldots t$; $i=1,\ldots n$. Where Y_{ijk} is the value of the dependent variable for the i^{th} animal for the j^{th} treatment on the k^{th} tubule; μ is the common mean effect; α_i is the effect of the i^{th} animal; τ_j is the effect of the j^{th} treatment; θ_{ij} is the interaction effect for the i^{th} animal on the j^{th} treatment, γ_{ik} is the effect of the k^{th} tubule of the i^{th} animal and ϵ_{ijk} is the random error assumed i.i.d. as N (O, σ^2).

The revised analysis of variance tables is included in this section (Table IV). (The notation and the details of calculation are being presented in a separate communication.)

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