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# THE EFFECT OF POTASSIUM ON INTRACELLULAR BICARBONATE IN SLICES OF KIDNEY CORTEX<sup>1</sup>

BY HELEN M. ANDERSON AND GILBERT H. MUDGE<sup>2</sup> WITH THE TECHNICAL ASSISTANCE OF THEODORA J. LANNON

(From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City, N. Y.)

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An increase in serum bicarbonate concentration has been observed in association with potassium depletion in a variety of clinical and experimental conditions. Although the development of the alkalosis may be extra-renal in origin, its perpetuation must depend upon an alteration in renal function which can be characterized as either an increase in bicarbonate reabsorption or an increase in hydrogen ion excretion. Many instances of so-called "paradoxical" aciduria have been described (cf. 1, 2).

Recent attempts to interpret this phenomenon have been predicated upon the concept that the extracellular alkalosis of potassium depletion is accompanied by an intracellular acidosis. Supporting evidence has been derived from a variety of experiments. In the muscle of potassium depleted rats Gardner, MacLachlan, and Berman (3) observed a decrease in the intracellular pH as calculated from whole tissue analyses corrected for extracellular space. Cooke and co-workers (4), on the basis of balance experiments and muscle analyses, concluded that the loss of intracellular potassium in rats was accompanied by an intracellular accumulation of both sodium and hydrogen ions. In studying experimental potassium depletion in man, Black and Milne (5) interpreted their balance data as indicating a shift of hydrogen ions into cells. In an attempt to obtain direct evidence relating the electrolyte composition of the renal parenchyma to the excretion of acid urine, Darrow, Cooke, and Coville (6) analyzed the kidneys of potassium depleted rats, but with inconclusive results.

Since the intracellular sodium and potassium

concentrations in the kidney cortex can be varied over a wide range by the use of *in vitro* techniques, studies of tissue slices were undertaken in order to define the relationships of intracellular potassium and bicarbonate.

## METHODS

The preparation of tissues has been described previously in detail (7) and is summarized here. Fresh slices were made from the renal cortex of rabbits killed by carotid exsanguination and were depleted of potassium by leaching for one hour in 0.15 N NaCl at room temperature. Half the slices were then incubated in Warburg flasks in 3 ml. of a medium containing NaHCO<sub>3</sub>, 18 mEq. per L., CaCl<sub>2</sub>, 1.3 mEq. per L., sodium phosphate buffer (pH 7.4) 3.7 mM. per L. and sodium acetate 0.01 mM. per L.<sup>3</sup> The remaining slices were incubated in a medium identical in composition except for the addition of 10 mEq. per L. of KCl. Sufficient NaCl was added to both media to provide constant osmolar concentrations of 300 mOs. per L. The Warburg vessels were gassed with a mixture of 5 per cent CO<sub>2</sub> and 95 per cent O<sub>2</sub>. The carbon dioxide-bicarbonate buffer system provided a pH of 7.4 in the external medium (8). Following incubation for 35 minutes at 25° C. the slices were promptly removed, blotted, and then analyzed for water and electrolyte. This entire procedure will be referred to hereafter as the *standard system*; modification of individual variables will be described for each special circumstance.

Tissue sodium and potassium were determined by flame photometry using lithium as an internal standard. In early experiments tissue chloride was determined by a modified Volhard titration. In later experiments, through the courtesy of Dr. Paul Marks, chlorides were measured potentiometrically using a silver-silver chloride cell (9).

"Acid-labile CO<sub>2</sub>" was determined by a modification of the technique devised by Conway and Fearon (10). Conway dishes (inner chamber 40 mm. diameter, 5 mm. high) were prepared with 1.3 ml. of 0.025 N Ba(OH)<sub>2</sub> in the center well, covered with ground glass cover-slips and sealed with anhydrous lanolin. After incubation in the Warburg flasks, tissue slices (about 300 mg. per

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<sup>2</sup> Present Address: Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore 5, Maryland.

<sup>3</sup> Although these amounts of calcium and phosphate were routinely added, their omission did not influence the results.

TABLE I

*Effect of potassium on tissue bicarbonate concentration*

	Tissue composition		
	HCO <sub>3</sub> <sup>-</sup>	K <sup>+</sup>	Na <sup>+</sup> + K <sup>+</sup>
	<i>mEq./Kg. wet weight</i>		
Before incubation	—	35.0	137
After incubation			
No K <sup>+</sup> added to external medium	13.5	34.9	138
10 mEq./L. K <sup>+</sup> added to external medium	18.0	77.2	135

vessel) were quickly transferred to the outer well of a prepared Conway dish. About 5 ml. of 2 N H<sub>2</sub>SO<sub>4</sub> was then added to the outer well on the side opposite the tissues. At all times the cover glass was slipped open only far enough to allow insertion of the sample or the pipette tip. The dish was promptly resealed and gently rotated to provide complete penetration of acid into the slices. The Conway dishes so loaded with Ba(OH)<sub>2</sub>, tissue, and H<sub>2</sub>SO<sub>4</sub> were allowed to stand at room temperature for one hour. A one ml. aliquot of fluid from the center well was then removed and its content of Ba(OH)<sub>2</sub> determined by titration with 0.005 N HCl, using thymol blue as indicator. Calculation by difference revealed the quantity of Ba(OH)<sub>2</sub> converted to BaCO<sub>3</sub> and hence the amount of "acid-labile CO<sub>2</sub>" present in the tissue. A Conway dish without tissue was run with each experiment as a control. Standardization of this technique using known concentrations of NaHCO<sub>3</sub> solution in the outer well gave 95 to 105 per cent recoveries. Longer standing, increased temperature, or gentle agitation of the Conway dish did not significantly alter the recovery. In one experiment, through the courtesy of Dr. Duncan Holaday, "acid-labile CO<sub>2</sub>" of the slices was measured by the gasometric method of Danielson and Hastings (11).<sup>4</sup> The results were the same as those obtained by the modified Conway technique.

All expressions for the concentration of electrolyte in tissues are given per kilogram wet weight. The qO<sub>2</sub> was calculated as the cubic millimeters of O<sub>2</sub> consumed per hour per mg. initial wet weight of tissue. Tissue bicarbonate determinations were done in duplicate on tissue incubated in separate flasks and treated identically. Tissue Na, K, and Cl were determined in duplicate on the specimen from a single flask.

<sup>4</sup> "Acid-labile CO<sub>2</sub>" is considered to include dissolved CO<sub>2</sub>, H<sub>2</sub>CO<sub>3</sub>, and HCO<sub>3</sub><sup>-</sup>. For simplicity, except as indicated in Table VIII, the term bicarbonate is used as a synonym for "acid-labile CO<sub>2</sub>" since this is by far the largest component of the CO<sub>2</sub>-H<sub>2</sub>CO<sub>3</sub>-HCO<sub>3</sub><sup>-</sup> system. It is recognized that Conway and Fearon (10) have presented evidence to suggest that compounds other than CO<sub>2</sub>-H<sub>2</sub>CO<sub>3</sub>-HCO<sub>3</sub><sup>-</sup> are measured as "acid-labile CO<sub>2</sub>." However, since the nature of these compounds is not clear and confirmation of this observation has not been presented, the conventions of Danielson and Hastings (11) have been adopted here.

## RESULTS

A typical experiment employing the *standard system* is illustrated in Table I. Before incubation, the potassium concentration of the leached slice was 35 mEq. per Kg. After incubation in a potassium-free medium there was no change in tissue potassium, but incubation in the medium containing 10 mEq. per L. of potassium resulted in an accumulation of potassium by the tissue to a final concentration of 77.2 mEq. per Kg. This value is similar to that found in fresh tissue (7). The uptake of potassium by the slices was associated with a loss of sodium, so that there was no significant change in the sum of sodium and potassium.<sup>5</sup> The bicarbonate concentration of the tissue incubated without potassium was 13.5 mEq. per Kg.; slices with added potassium had a significantly higher bicarbonate concentration (18.0 mEq. per Kg.).

TABLE II

*Average values from 17 experiments employing the Standard System\**

	Final tissue composition			
	K <sup>+</sup> added to incubation medium		Δ ± S.D.	P
	0	10 mEq./L.		
HCO <sub>3</sub> <sup>-</sup> , <i>mEq./Kg.</i>	12.3	17.0	4.7 ± .52	<.001
K <sup>+</sup> , <i>mEq./Kg.</i>	32.1	71.7	39.6 ± 1.2	<.001
Na <sup>+</sup> + K <sup>+</sup> , <i>mEq./Kg.</i>	140	141	1.0 ± 1.5	>.5
H <sub>2</sub> O, % <i>wet weight</i>	77.8	78.7	0.9 ± .24	<.001

\* Eight analyses of fresh kidney cortex, removed as quickly as possible after killing the rabbit, yielded an average bicarbonate concentration of 14.4 mEq. per Kg. Fresh tissue values previously reported (7) are: K<sup>+</sup>, 69.3 mEq. per Kg.; Na<sup>+</sup> + K<sup>+</sup>, 138 mEq. per Kg.; H<sub>2</sub>O, 77.1 per cent wet weight.

A similar relationship was observed in a total of 72 experiments. In Table II are summarized the results from 17 experiments in which tissue bicarbonate, sodium, potassium and water content were measured. The average difference in tissue bicarbonate between the slices with and without added potassium was 4.7 mEq. per Kg.; this was associated with an average change of 39.6 mEq. per Kg. in the level of tissue potassium.

<sup>5</sup> In this presentation the convention of correlating tissue bicarbonate to potassium will be employed. It is recognized that because of the nature of the cation changes, tissue sodium could be used as a reference equally well.

The sum of tissue sodium and potassium remained constant, although small changes may have escaped detection because of difficulties inherent in the measurement of tissue sodium. The accumulation of potassium was associated with a small, but statistically significant, increase in tissue hydration. As will be noted in subsequent tables, the absolute level of tissue bicarbonate varied somewhat from one experiment to another, but the difference between the slices with low and high tissue potassium remained constant. In every experiment, therefore, suitable internal controls were employed.

The dependence of tissue bicarbonate on the intracellular accumulation of potassium rather than on the potassium concentration in the incubation medium is illustrated by experiments in which respiration or aerobic phosphorylation was inhibited. Previous studies (7) have shown that the uptake of potassium is related to aerobic metabolic activity. The effects of anaerobic incubation

TABLE III  
Comparison of aerobic and anaerobic incubation \*

Gas	Medium K <sup>+</sup>		Tissue	
	Initial	Final	HCO <sub>3</sub> <sup>-</sup>	K <sup>+</sup>
	mEq./L.	mEq./L.	mEq./Kg.	mEq./Kg.
5% CO <sub>2</sub> -95% O <sub>2</sub>	0	0	14.4	41.3
	10	8.4	17.3	75.3
5% CO <sub>2</sub> -95% N <sub>2</sub>	0	2.6	10.6	15.3
	10	12.7	10.9	24.5

\* Before incubation the tissue contained 42.4 mEq. K<sup>+</sup> per Kg.

are illustrated in Table III. The experimental conditions were the same as those of the *standard system* except that the gas mixture was 5 per cent CO<sub>2</sub>-95 per cent N<sub>2</sub> instead of 5 per cent CO<sub>2</sub>-95 per cent O<sub>2</sub>. The anaerobic tissues showed a net loss of potassium and a tissue bicarbonate concentration unchanged by the addition of potassium to the external medium.

It has been shown that 2,4-dinitrophenol uncouples aerobic phosphorylation and prevents the uptake of potassium by kidney slices (12). As shown in Table IV, this agent causes a failure of bicarbonate accumulation and a parallel inhibition of potassium uptake.

Previous observations have shown that the presence of low concentrations of potassium in the external medium increases the rate of oxygen

TABLE IV  
Effect of 2,4-dinitrophenol \*

2,4-DNP M	Medium K <sup>+</sup>		Tissue composition	
	Initial	Final	HCO <sub>3</sub> <sup>-</sup>	K <sup>+</sup>
	mEq./L.	mEq./L.	mEq./Kg.	mEq./Kg.
0	0	0	13.3	40.2
0	10	6.8	20.5	74.8
3 × 10 <sup>-5</sup>	10	9.8	16.7	42.0
9 × 10 <sup>-5</sup>	10	11.6	12.7	28.8
3 × 10 <sup>-4</sup>	10	11.9	12.7	25.3

\* Before incubation the tissue contained 35.9 mEq. K<sup>+</sup> per Kg. The sum of Na<sup>+</sup> plus K<sup>+</sup> remained constant.

consumption (7). This suggested that the change in the tissue level of bicarbonate might be a direct effect of the respiratory stimulation produced by the addition of potassium. The use of the indirect Warburg technique (8) permitted the determination of the qO<sub>2</sub> in the presence of CO<sub>2</sub> in the gas phase. Experiments were carried out with 40 mEq. per L. of potassium in the incubation medium, instead of 10 mEq. per L., since this higher concentration is not generally accompanied by respiratory stimulation. The results are shown in Table V, and clearly indicate that the change in bicarbonate in tissues incubated with added potassium is not dependent upon increased oxygen consumption. In addition, in the earlier experiment with 2,4-dinitrophenol, despite marked respiratory stimulation there was a fall, rather than an increase, in the level of tissue bicarbonate. The substitution of succinate, citrate or α-ketoglutarate for acetate in the *standard system* did not change the tissue level of either bicarbonate or potassium. With succinate as substrate there was approximately a fifty per cent increase in oxygen consumption. It is concluded, therefore, that the effect of potassium on tissue bicarbonate is not the direct result of respiratory stimulation.

The influence of different levels of tissue potassium on bicarbonate was examined over a wide range by varying the amount of potassium chloride added to the medium. The osmolarity was kept constant by adjustments in sodium chloride. The results of four separate experiments are illustrated in Figure 1. It is seen that tissue bicarbonate and potassium are linearly related except at the very high tissue potassium levels, which were achieved by raising the external concentration to as high as 120 mEq. per L. The results of high external concentrations are difficult to evaluate be-

TABLE V  
Relation of oxygen consumption to tissue bicarbonate\*

Medium K <sup>+</sup> mEq./L.	qO <sub>2</sub>	Tissue HCO <sub>3</sub> <sup>-</sup> mEq./Kg.
0	.63	9.3
40	.54	15.7

\* The qO<sub>2</sub> was measured by the indirect method of Warburg (8).

cause of marked respiratory depression and excessive tissue hydration.

The rise in tissue bicarbonate noted above could result from either an increase in the total number of anions or a replacement of some anions by bicarbonate. Evidence supporting the latter mechanism is afforded by two observations: 1) The sum of tissue sodium and potassium remained constant (see Table II); and 2) the changes in tissue chloride which were observed in a series of experiments (Table VI). The average change upon the addition of potassium was +4.0 mEq. per Kg. of bicarbonate and -5.7 mEq. per Kg. of chloride. Within the limits of the methods employed, these changes are considered to be approximately equivalent. The substitution of nitrate for chloride in the external medium resulted in a fall in tissue chloride to about 6 mEq. per Kg. The tissue bicarbonates in the nitrate medium were the same as in the chloride medium, and the usual change in tissue bicarbonate was produced by the addition of potassium. The results are interpreted as evidence that the changes in tissue chlo-

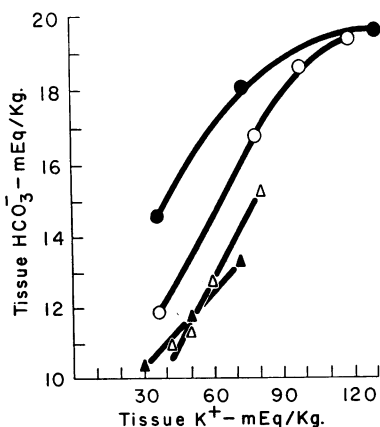


FIG. 1. RELATION OF TISSUE BICARBONATE TO TISSUE POTASSIUM

Lines indicate four separate experiments. Tissue potassium was varied by changing external concentration.

ride (cf. Table VI) associated with the accumulation of potassium are secondary to changes in tissue bicarbonate.

In a number of biological systems it has been reported that rubidium and cesium behave in a manner similar to potassium. As shown in Table VII the addition of the chloride salts of these elements to the incubation medium resulted in changes in tissue bicarbonate resembling those produced by potassium, whereas lithium was ineffective. It is of interest that hypokalemic alkalosis in the rat may be corrected by the administration of rubidium (13). However, the present results with lithium are not what might be anticipated from previous studies in the dog in which

TABLE VI  
Relation of tissue chloride and bicarbonate\*

HCO <sub>3</sub> <sup>-</sup> , mEq./Kg.			Cl <sup>-</sup> , mEq./Kg.		
No K <sup>+</sup> added	K <sup>+</sup> added	Δ ± S.D.	No K <sup>+</sup> added	K <sup>+</sup> added	Δ ± S.D.
15.2	19.2	+4.0 ± .41	64.9	59.2	-5.7 ± .52

\* Values reported are averages from eight experiments in which the standard system was employed. Chloride was determined by a modified Volhard titration in four experiments, and by a potentiometric method (9) in the remainder. Similar results were obtained by both methods. The chloride concentration in the incubation medium was, initially, 118 mEq. per L.

it was shown that potassium excretion and urine pH were increased by the administration of this ion (14, 15).

Studies of the effect of changes in the external concentration of bicarbonate were undertaken and the results of a typical experiment are given in Table VIII. In this and five similar experiments, pCO<sub>2</sub> was maintained constant so that the alterations in external bicarbonate were associated with parallel changes in the pH of the medium. The phosphate buffer used in the standard incubation medium was omitted. At low concentrations of external bicarbonate the addition of potassium had a much greater effect on the level of tissue bicarbonate than in the standard system. Conversely, at high external bicarbonate concentrations, the effect of potassium was negligible. The pH of the external solution had no effect on the final tissue concentration of potassium or of potassium plus sodium, either with or without the addition of potassium to the medium. Similarly,

respiration and tissue hydration were essentially the same at each pH.

Because of the changes in external bicarbonate, the data from these experiments can not be evaluated solely in terms of total tissue concentrations. Therefore, intracellular values were calculated on the basis of the assumptions listed in Table VIII. Despite recognized limitations, these data probably represent a reasonable approximation and afford a valid basis for comparison. As shown in Figure 2, in the potassium depleted slice the intracellular concentration of bicarbonate rises as the external level is increased. In contrast, slices with normal tissue potassium maintain nearly con-

TABLE VII  
Effect of other cations \*

Salt added	Tissue HCO <sub>3</sub> <sup>-</sup> mEq./Kg.
Sodium	14.9
Lithium	15.0
Potassium	19.1
Rubidium	18.4
Cesium	20.5

\* Ten mEq. per L. of the chloride salt of the indicated cations was added to the external medium. Total osmolarity was kept constant by the addition of sodium chloride in a concentration of 105 mEq. per L., which, plus the other cup components, provided a total osmolar concentration of 300 mOs. per L. (See Methods.) The ions listed above had no significant effect on the rate of oxygen consumption.

stant intracellular bicarbonate despite a five-fold change in external concentration.

DISCUSSION

These results directly demonstrate that the level of tissue bicarbonate can be influenced by potassium. For reasons previously mentioned these effects may be attributed to intracellular changes. If applied to certain physiological adjustments which are observed in the intact animal, the findings could provide an explanation for the "paradoxical" aciduria of potassium depletion. Recent studies indicate that the exchange of sodium from the tubular urine for hydrogen ion from the tubule cell is the mechanism by which bicarbonate is reabsorbed and the urine acidified (16-18). Berliner, Kennedy, and Orloff (19) have postulated that the sodium-potassium exchange responsible for the tubular secretion of potassium may compete with

TABLE VIII

Effect of external bicarbonate on calculated intracellular pH \*

	External		Tissue HCO <sub>3</sub> <sup>-</sup> mEq./Kg.	"Intracellular"	
	HCO <sub>3</sub> <sup>-</sup> mEq./L.	pH		HCO <sub>3</sub> <sup>-</sup> mEq./L. ICW	pH
No K <sup>+</sup> added	5	6.85	7.1	11	7.00
	18	7.40	13.3	16.5	7.18
	28	7.60	21.0	26.4	7.38
K <sup>+</sup> added 10 mEq./L.	5	6.85	15.8	27.3	7.40
	18	7.40	19.9	29.1	7.42
	28	7.60	22.4	29.1	7.42

\* All cups were gassed with 5 per cent CO<sub>2</sub>-95 per cent O<sub>2</sub>. The pH of the medium was calculated from the nomogram of Umbreit, Burris, and Stauffer (8). The intracellular values were calculated by the method of Wallace and Hastings (21), assuming: 1) tissue solids, 22 per cent of total tissue; 2) extracellular space, 25 per cent of total tissue; 3) pK' for carbonic acid, 6.1; 4) the same pCO<sub>2</sub> in the intracellular water as in the external medium; 5) solubility coefficient of CO<sub>2</sub> in the water of the intracellular space, 0.592; 6) all acid-labile tissue CO<sub>2</sub> to be bicarbonate ion.

sodium-hydrogen exchange. For example, the aciduria of potassium depletion might be attributed to a decrease in the rate of potassium secretion and an increase in sodium-hydrogen exchange. The present results may define some of the regulatory factors more precisely, namely that the apparent competition between hydrogen and potassium ions for an excretory mechanism may be a reflection of their relative intracellular concentrations. Thus, the internal environment of the tubule cell may directly modify the composition

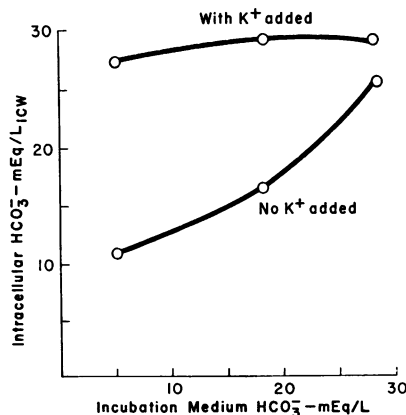


FIG. 2. RELATION OF INTRACELLULAR BICARBONATE TO EXTERNAL BICARBONATE CONCENTRATION

All tissues gassed with 5 per cent CO<sub>2</sub>-95 per cent O<sub>2</sub>. For initial pH values of media, see Table VIII.

of the urine, and extra-renal regulatory mechanisms need not be implicated.

In their studies on rat skeletal muscle, Cooke, Segar, Cheek, Coville, and Darrow (4) suggested that during the development and repair of hypokalemic alkalosis the quantitative relationships between intra- and extra-cellular univalent cations might be described by an ionic exchange in the ratio of three potassiums for two sodiums and one hydrogen. Because of the difficulties inherent in the estimation of intracellular buffer capacity, the calculation of this ratio is only an approximate description. In the present experiments, at an external pH of 7.4, an exchange of eight sodium for eight potassium ions is accompanied by a simultaneous exchange of one chloride for one bicarbonate ion. Estimates of changes in intracellular hydrogen ion concentration in this system have only limited validity because of the following factors: 1) The exact nature of intracellular "acid-labile" carbon dioxide has not been ascertained (10); and 2) calculations presented above for the kidney cortex have assumed a homogeneous cell population. If the cells do not behave uniformly, the calculated changes would be smaller in some cells and greater in others.

Despite these limitations, the derived intracellular pH suggests that the level of potassium within the cells is related to their buffer capacity. As shown in Table VIII and Figure 2, a normal potassium concentration is associated with only small changes in intracellular pH when the external pH is varied. However, the results do not indicate an absolutely constant relationship between intracellular potassium and hydrogen ion concentrations. This is shown by the results obtained with the potassium depleted slice (Figure 2). Although the level of intracellular potassium remains constant, the pH of these slices is markedly influenced by the pH of the external environment.

While some of the observations reported here are qualitatively similar to those predicted by the Boyle-Conway (20) application of the Donnan equilibrium, the quantitative relationships between intra- and extra-cellular ions deviate so far from the predicted values that the hypothesis need not be considered further. An interpretation of the present results on a physico-chemical basis is not warranted at this time.

#### SUMMARY

Studies on slices of rabbit kidney cortex have directly demonstrated that the intracellular concentration of bicarbonate is related to that of potassium. These findings support the hypothesis that the pH of the cells of the renal tubules may directly influence renal excretion in the regulation of acid-base balance in conditions of potassium depletion or excess.

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