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THE RÔLE OF THE KIDNEY IN THE REGULATION OF ACID-BASE BALANCE

It would be presumptuous to ascribe a major interest to John Peters. His field was all of human physiology. And yet it would not be remiss to consider his contribution to the understanding of acid-base balance as among his most significant efforts. Today, 25 years later, little can be added to the exposition of CO_2 transport in blood, the nature of buffers, and the reaction to acidosis and alkalosis as described in *Quantitative clinical chemistry.*[87] The rôle of the kidney in the maintenance of the electrolyte equilibrium of the body was only beginning to be understood in the 1930's and much has transpired since the publication of Peters' early papers. His continuing interest in this aspect of investigation can be measured in part by the number of his former associates and students who, stimulated and prodded by his penetrating questions, have concerned themselves with renal physiology.

It will be the purpose of this paper, not to review in detail the mechanism of the renal regulation of acid-base balance—this has been the subject of several excellent papers[19, 88, 89]—but rather to discuss a few of the more recent contributions in an effort to delineate some of the problems, emphasizing those of a controversial nature.

THEORIES OF ACIDIFICATION

The primary function of the kidney in the regulation of acid-base balance is to maintain electrolyte equilibrium. Thus, although acute disturbances of acid-base balance, whether of metabolic or respiratory origin, are automatically countered by buffers of blood and tissues and by variations in the excretion of CO_2 by the lungs, excesses and deficits of fixed cation or anion must in the final analysis be corrected by differential excretion by the kidney.

Urinary acidification is characterized by the excretion of both titratable acid and ammonium salts. In Peters' earlier years it was thought that all of the excreted acid was originally present in the glomerular filtrate and that net acidification was achieved through preferential reabsorption of the

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alkaline moiety of the urinary buffer pairs (Na_2HPO_4/NaH_2PO_4 and $NaHCO_3/H_2CO_3$). The substitution of ammonium chloride for sodium chloride was thought to be an independent process.

Pitts and his associates in a series of classical papers demonstrated the inadequacy of this formulation by showing that neither bicarbonate nor dibasic phosphate reabsorption could account for the high rates of titratable acid excretion noted in metabolic acidosis.[40, 41, 42] They concluded, as Smith had postulated before them,[51] that hydrogen ions must be added to urine in excess of that filtered, and considered this to be accomplished by extrusion of hydrogen ions into tubule urine in exchange for filtered sodium. This process accounts quantitatively for the excretion of titratable acid and of ammonia and for the reabsorption of bicarbonate in the following manner: (a) titration of alkaline buffer salts to their acid form thereby increasing titratable acid excretion (e.g., $Na_2HPO_4 + H^+ \rightarrow NaH_2PO_4$); (b) establishment of a hydrogen ion gradient* between cells and urine with resultant accumulation of ammonium ion; (c) interaction of secreted hydrogen ion with urinary bicarbonate ion, formation of carbonic acid, and its dissipation as CO_2 and water. In all of the above processes sodium and bicarbonate are ultimately transported into the blood. Figure 1, modified from Pitts, summarizes the salient features of this theory. The rôle of the carbonic anhydrase system differs from that postulated by Pitts and will be discussed below.

Despite general acceptance of this view of urinary acidification, it must be recognized that there is no direct evidence for the existence of an ion exchange process in renal tubule cells. Furthermore, at least two other theories are consistent with the experimental observations. Menaker, in Peters' laboratory, pointed out that reabsorption of carbonate ion from glomerular filtrate will account for observed rates of hydrogen ion excretion.[30] However, since considerable uptake of carbonate, an ion present in vanishingly low concentration in urine, would be necessary to effect maximal rates of hydrogen ion excretion, Pitts considers this mechanism improbable.[39] His objection, though valid, is not necessarily critical. More recently Brodsky has postulated that sodium bicarbonate reabsorption in an area permeable to CO_2 is equally compatible with the data.[10] It is his view that sodium bicarbonate may be reabsorbed by an active process. The resultant fall in pH would lead to the interaction of carbonic acid with nonbicarbonate buffers forming titratable acid and more sodium bicarbonate. CO_2 from blood would then diffuse into urine along the new concentration

* The maximal H^+ ion gradient between plasma and urine is approximately 1000 : 1, that between cells and urine is unknown.

gradient, interact with water to form carbonic acid and thereby serve as a continuing source of hydrogen and bicarbonate ions. Although all degrees of urinary acidification could be achieved by a cyclical process of this nature, the hypothesis has a number of limitations. In the first place it requires that both sodium and bicarbonate ions be reabsorbed by active processes, rather than sodium alone as in Pitts' hypothesis. Though a positive intraluminal electrical potential difference produced by bicarbonate ion abstraction could effect the passive movement of sodium out of urine, this is unlikely in view of available measurements of lumen potential, all of which have been distinctly negative.[52] Furthermore, Berliner has pointed out that the rate-limiting reaction in Brodsky's system is in all probability the hydration of CO_2 in urine.[8] The speed of this reaction is insufficient in the absence of carbonic anhydrase to account for observed rates of acid excretion in metabolic acidosis.* Despite these objections, it must be reiterated, as with the Menaker hypothesis, that none is sufficient in itself to exclude the existence of the postulated mechanism. However, there is good evidence for specific and active reabsorption of sodium in the kidney. The specificity, as Ussing has pointed out, is strongly suggestive of transport by combination with a carrier.[55] Since this is most readily visualized as accomplished by a process of ion exchange, Pitts' hypothesis represents the most reasonable concept of urinary acidification.

One of the findings interpreted as supporting the view that hydrogen ion is secreted into urine is the elevation of the CO_2 tension of alkaline urine above that of plasma. The precise mechanism of this rise has been the subject of considerable debate.[15, 24, 32, 41] Pitts has assumed that the excess CO_2 in urine is derived from carbonic acid formed by the secretion of hydrogen ion into bicarbonate containing tubule fluid (Fig. 1 (c)). Since renal tubule cells are believed to be freely permeable to dissolved CO_2, observed elevations in the CO_2 tension of urine (the sum of dissolved CO_2 + carbonic acid) are ascribed to the delayed dehydration of this carbonic acid in the absence of carbonic anhydrase.[41] Mainzer[27] and Clark,[11] on the other hand, assumed that elevations in urine CO_2 tension above plasma were due to admixture in the bladder of urines formed at different times and of differing pH, the excess CO_2 being produced by titration of the bicarbonate of the less acid urine. Kennedy, Orloff, and Berliner were able to exaggerate

* It may be recalled that the equilibrium ratio of the concentration of dissolved CO_2 to carbonic acid in the body is 800: 1. Only carbonic acid interacts with buffers. The speed of attainment of equilibrium in the absence of carbonic anhydrase is slow, requiring 200 seconds to come within 10 per cent of equilibrium at 38° C.[48] Though urine does not contain appreciable amounts of carbonic anhydrase, the possibility that the enzyme is present in the luminal membrane cannot be excluded.

the difference between the CO_2 tensions of urine and plasma by increasing the concentration of buffer in urine.[24] They suggested that urine pH differs in individual tubules and that admixture occurs above the renal pelvis. The surface-volume relationship in this area was thought to be unfavorable for free diffusion of CO_2 into plasma so as to account for the observed gradient.

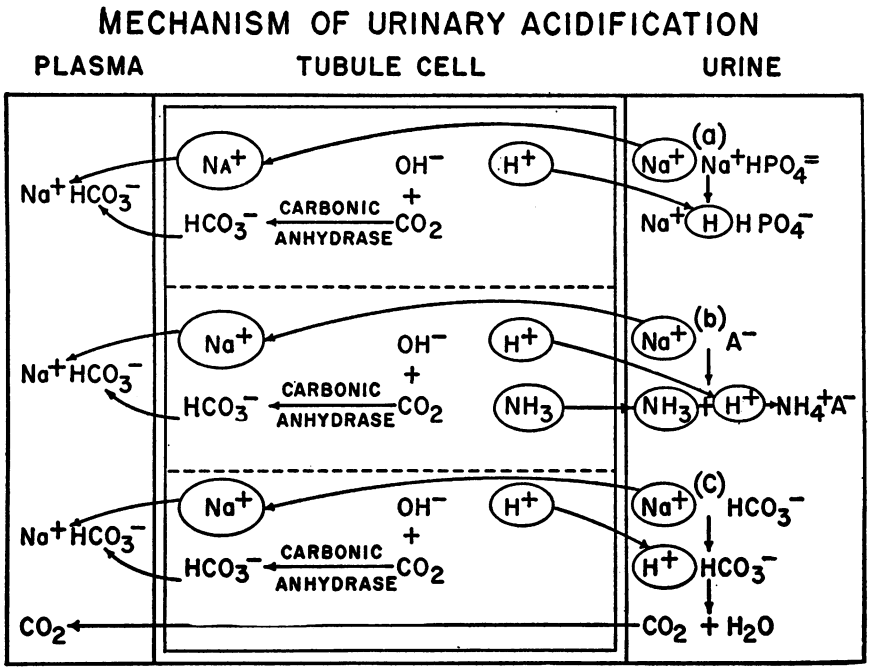

FIG. 1. (a) Formation of titratable acid, (b) formation of NH_4^+, (c) reabsorption of filtered bicarbonate. Carbonic anhydrase is not depicted as involved directly in the transport process; it catalyzes either the interaction between CO_2 and OH^- or the hydration of CO_2. In the latter instance H_2CO_3 can then neutralize OH^-.

Added evidence for the mixing hypothesis was adduced by varying urine flow, in which instance urine CO_2 tension diminished with increasing flow due to the lowering of buffer concentration. This is counter to the delayed dehydration hypothesis since a decrease in urine transit time would further diminish the time available for the dehydration of carbonic acid.

Recently Ochwadt and Pitts demonstrated that an elevated CO_2 tension of urine may be reduced to the level of that of plasma by addition of carbonic anhydrase to urine through its intravenous administration.[32] While indicating that the delayed dehydration of carbonic acid does play a part in the elevation of urine CO_2 tension and, indeed, that the excess carbonic acid

involved is formed in an area permeable to CO_2, these experiments in no way define the mechanism of the formation of the carbonic acid. The elevation of CO_2 tension produced by mixing buffered urines of differing pH also involves carbonic acid as an intermediate in the process. The interpretation that the excess carbonic acid is formed by hydrogen ion secretion leaves unexplained the effect of urine buffer concentration, the effect of urine flow, and the reported observation of urine CO_2 tensions below those of plasma in acid urines of low buffer content. It is more likely that excess carbonic acid is formed by the mixing of urines of disparate pH in a region of tubule permeable to CO_2 (e.g., collecting tubules, collecting ducts). Under such circumstances addition of carbonic anhydrase would accelerate the dehydration of carbonic acid to CO_2 and water and reduce the CO_2 tension to that of plasma.

SOURCE OF URINARY HYDROGEN ION AND THE RÔLE OF THE CARBONIC ACID SYSTEM

It is widely held that hydrogen ion secreted into the urine is derived from intracellular carbonic acid formed by hydration of CO_2.[38, 40, 47] There is no evidence for this hypothesis and it may represent a misleading oversimplification. Data relating changes in plasma CO_2 tension and urinary acidification afford no information as to the source of extruded hydrogen ion.[8, 16, 45] Though intracellular carbonic acid concentration rises when plasma CO_2 tension is elevated, it cannot be determined whether this provides more substrate for the acidification process or exerts an effect by lowering cell pH. Similarly, experiments relating renal CO_2 production and hydrogen ion excretion are not pertinent in defining the origin of urinary acid.[47] CO_2 undoubtedly plays a rôle in the acidification process insofar as acid-base balance is concerned since, as noted in Figure 1, bicarbonate is formed whenever ammonia or titratable acid is excreted or bicarbonate reabsorbed. However, there may be appreciable secretion of hydrogen ions in the absence of acidification.* Thus it has been suggested that the great bulk of filtered sodium is reabsorbed by exchange with hydrogen ion.[2] Since urine pH does not change in the proximal segment, sodium may either exchange with hydrogen and chloride with hydroxyl ion or alternatively chloride be passively reabsorbed as a consequence of a negative electrical potential

* The actual turnover of hydrogen ions in cells and urine is of no consequence insofar as acid-base balance is concerned. Of importance is the net loss of acid from the body, estimated as the sum of the rates of excretion of titratable acid plus ammonia minus that of bicarbonate. The distinction between net acidification and rate of hydrogen ion secretion is of value, however, in evaluating the significance of a number of situations. Changes in acid excretion and hydrogen ion extrusion do not necessarily parallel each other and it is frequently difficult to estimate the relative rates of the latter on the basis of urinary data alone.

induced by active sodium transport followed by back diffusion of hydrogen ion. In either instance, so long as a steady state is maintained, neither luminal nor cell pH will change, nor will bicarbonate ion be produced.

Secreted hydrogen ion may be derived from other metabolic reactions. The rapid extrusion of hydrogen ion from cells would lead to a rise in cell pH and consequent interference with acidification, except in the isohydric segment as noted above, unless hydroxyl were disposed of first by cellular buffers and then by transport across the contraluminal border. The carbonic acid system in effect may serve both purposes, a ready source of disposable buffer minimizing cell pH changes and an indirect mode of hydroxyl transport. Davies and Roughton have commented on the rôle of CO_2 as a buffer with respect to the stomach,[18] and Jacobs and Stewart on that of bicarbonate as an hydroxyl transport system in red cells.[28] In this view, carbonic anhydrase enhances the buffering efficacy of the $CO_2/H_2CO_3/HCO_3^-$ system by catalyzing the formation of bicarbonate from CO_2 and hydroxyl ion; bicarbonate may then be transported into plasma.* This is depicted in Figure 1. Inhibition of carbonic anhydrase will interfere with acidification by diminishing the efficiency of the CO_2 buffer system, thereby allowing the development of intracellular alkalosis. Parenthetically, it may be stated that it matters little what the ultimate source of urinary hydrogen ion is insofar as net acid excretion is concerned. Whether it be derived from the hydration of metabolic CO_2 or from any other hypothetical process, in either instance, the rate limiting step in the acidifying process is the hydration of CO_2. However, the recognition that CO_2 probably serves essentially the same function in tubule cells as in other systems (e.g., red cells) is of importance.

THE SOURCE OF CO_2

Roberts *et al.* noted that the maximal rate of hydrogen ion excretion (defined by them as the sum of titratable acid plus ammonia excretion) in the dog approximated but never exceeded total renal CO_2 production.[47] They interpreted this as indicating that locally produced CO_2 is involved in the acidification process. However, the data actually constitute convincing evidence that CO_2 utilized in the secretory process must originate from other sources as well. Since only a fraction of renal cells is engaged in the formation of titratable acid and ammonia, CO_2 of necessity must be added to these cells from the plasma to account for the observations. Furthermore, their definition of hydrogen ion secretion omits from consideration hydro-

* Whether carbonic anhydrase catalyzes the reaction
$CO_2 + H_2O \rightleftharpoons H_2CO_3$ or
$CO_2 + OH^- \rightleftharpoons HCO_3^-$ is of no consequence since in the first instance the dissociation of H_2CO_3 is instantaneous, making a distinction between the processes impossible.

gen ion expended in converting filtered bicarbonate to carbonic acid. Much more hydrogen ion may be used in this process than in the formation of titratable acid or ammonia. Consequently this would impose on the secretory cells an even greater requirement for metabolic CO_2 production. Urinary carbonic acid derived from the interaction of hydrogen and bicarbonate ions in the reabsorption of bicarbonate cannot contribute CO_2 to the pertinent cells inasmuch as the dehydration of carbonic acid presumably occurs in an area distal to the acidifying segment.

TABLE 1. RELATIONSHIP BETWEEN INTRACELLULAR HYDROGEN AND POTASSIUM CONCENTRATION AND URINARY ACIDIFICATION

	Cell		*Urinary excretion*				*Urine*
	pH	*K+*	*T.A.**	*NH_3*	*K+*	*HCO_3^-*	*pH*
Respiratory acidosis	Decr.†	Norm.‡	Incr.§	Incr.	Decr.	Decr.	Decr.
Hypokalemic alkalosis	Decr.	Decr.	Incr.	Incr.	Decr.	Decr.	Decr.
Respiratory alkalosis	Incr.	Norm.	Decr.	Decr.	Incr.	Incr.	Incr.
Potassium infusion	Incr.	Incr.	Decr.	Decr.	Incr.	Incr.	Incr.
Acetazolea-mide admin-istration	Incr.	Norm. or Decr.	Decr.	Decr.	Incr.	Incr.	Incr.

* Titratable acid. † Decrease. ‡ Normal. § Increase.

FACTORS INFLUENCING THE SECRETION OF HYDROGEN AND POTASSIUM IONS

Despite limited information concerning either the source of hydrogen ion or its mode of transport into urine, a number of factors are known which influence its rate of secretion. Furthermore, since potassium is also secreted,[4, 81] presumably in exchange for sodium, it is not surprising that factors which influence acidification may also exert effects on the excretion of potassium. The intracellular concentration of hydrogen ion appears to be an important determinant of the secretory rates of these ions.[2, 5, 85] Factors which can be assumed to decrease cell pH enhance the excretion of titratable acid and ammonia and diminish that of potassium and bicarbonate. The converse is observed when cell pH is presumably elevated. The relationship between cell hydrogen ion content and the respective urinary changes for a number of situations to be discussed below is summarized in Table 1. The intracellular content of hydrogen and potassium in renal tubule cells has not been determined analytically, but assumed on the basis of inferential evidence. It should be noted that the urinary changes are elicited even when cell potassium content is presumably normal. Though the reciprocal

changes in the excretion of potassium and hydrogen ions observed in hyper- and hypokalemia may be conditioned largely by variations in intracellular potassium content, changes in cell pH induced by transcellular shifts of potassium and hydrogen ions may also play an important rôle. In any event, cell pH is thought to be altered in all of the situations listed in Table 1, whereas cell potassium content is presumably unchanged in the respiratory modifications of acid-base balance and during inhibition of carbonic anhydrase activity.

The importance of the filtered load of bicarbonate and nonbicarbonate buffers in the process of acidification cannot be minimized. The composition of glomerular filtrate in this regard is obviously a major factor insofar as net acidification is concerned. It may affect the secretory rates of hydrogen and potassium ions apparently in the absence of changes in the cell content of either ion; whether the filtered load exerts an independent influence on the maximal hydrogen ion gradient that can be established between urine and plasma is unknown. Net transport of hydrogen ion apparently ceases when urine pH approximates 4.5, but the mechanism is unclear.[88] Maximal pH on the other hand need not be produced by secretion of alkali but may be explained by concentration of urine bicarbonate. Thus abstraction of water from bicarbonate containing urine increases the concentration of bicarbonate to a greater extent than that of the diffusible CO_2, consequently pH's above that of plasma may be achieved.

PLASMA CO_2 TENSION AND ACIDIFICATION

The relationship between plasma CO_2 tension and acidification has been commented on before[85] and experimentally defined by a number of workers.[8, 16, 45] Though the changes have been ascribed to alterations in the carbonic acid content of renal tubule cells, it has not been possible to exclude with certainty the influence of variations in total body acid-base balance on the urinary findings. This has now been accomplished and the rôle of the kidney delineated in a more precise fashion by utilizing the chicken as an experimental animal.[84] Chickens possess a functioning renal-portal circulation.[58] Blood from the leg vein drains directly into the peritubule area of the same side, then enters the systemic circulation via the inferior vena cava, thereby bypassing the glomerulus in the first circulation. Catheterization of the individual ureteral openings in the cloaca permits separate collection of urine from each kidney. Because of this unique situation it is possible to investigate agents which exert effects on tubule cells alone by administering the substance in one leg and comparing urine from the kidney of the infused side with that from the contralateral kidney which serves as a control.[58]

In order to examine the effect of alterations in renal CO_2 tension on acidification, changes in peritubule plasma CO_2 tension were produced by the addition of acid or alkali to saphenous vein blood. The details of one such experiment are summarized in Figure 2. Augmented potassium excre-

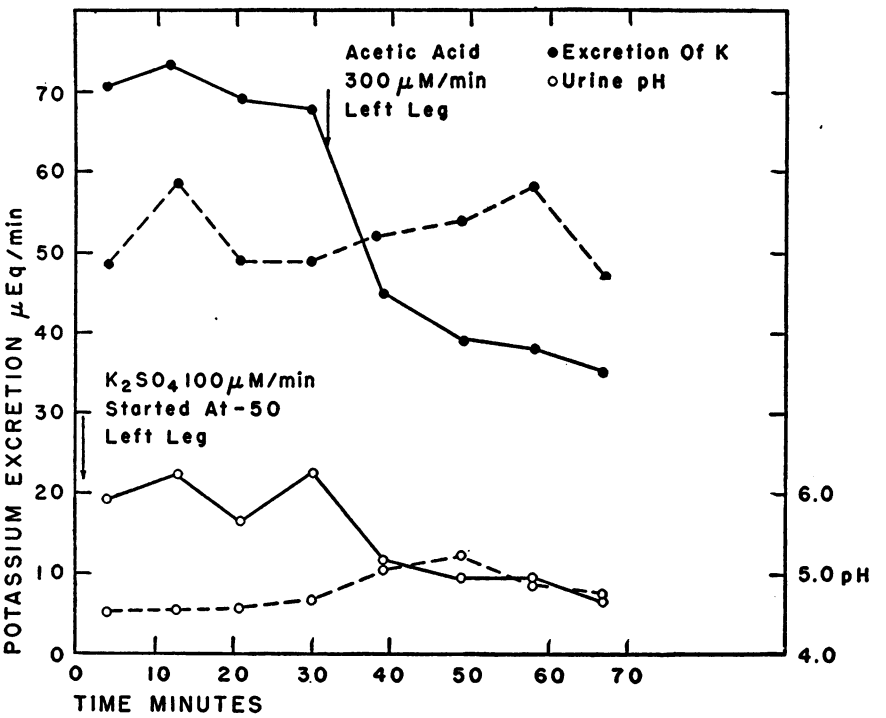

FIG. 2. The effect of elevation of plasma CO_2 tension on urine pH and potassium excretion. Data from the control kidney are illustrated by broken lines, from the experimental kidney by solid lines.

tion and a moderate decrease in hydrogen ion excretion were induced by the administration of potassium sulfate throughout the experiment. Acetic acid was then added to the blood perfusing the left peritubule area. This, by interaction with sodium bicarbonate, elevates plasma CO_2 tension in the immediate area and, since CO_2 diffuses freely, increases intracellular hydrogen ion concentration. The immediate fall in potassium excretion and rise in urine hydrogen ion concentration on that side are apparent. The transformation of acetate to bicarbonate obviates the development of metabolic acidosis. The experiment illustrates two points: (a) elevation of intracellular hydrogen ion and/or CO_2 tension augments hydrogen ion secretion,

and (b) secretion of potassium is inhibited, even in the absence of a fall in the concentration of potassium in the plasma and, presumably, in renal cells. The data are analogous to those observed in experimental and clinical respiratory acidosis in which titratable acid and ammonia excretion rises as excretion of potassium and bicarbonate falls.[16, 17] The converse, reduction of plasma CO_2 tension, was induced by the addition of sodium hydroxide to the saphenous blood. Interaction with carbonic acid produces a diminution in plasma CO_2 tension and resultant interference with acidification. Similar observations have also been made in metabolic alkalosis.[29, 50, 54] In the chicken experiments, it seems clear that the renal excretion of hydrogen and potassium ions can be affected by modifications of intracellular concentration of hydrogen ion alone and that alterations in systemic acid-base balance are not necessary prerequisites for these effects.

The effect of variations in plasma CO_2 tension on urinary acidification is of interest in another regard. The compensatory fall in plasma CO_2 tension in metabolic acidosis resulting from augmented respiratory exchange might be expected to interfere with hydrogen ion secretion. At first glance this appears paradoxical and contrary to bodily needs. Actually, net acidification is augmented in ammonium chloride acidosis, even though the rate of hydrogen ion transfer into urine may be depressed or at least unchanged (see above). The explanation for this phenomenon is obvious when one recognizes that normally a large fraction of secreted hydrogen ion is dissipated in the reabsorption of sodium bicarbonate. This may be in excess of that required for titratable acid and ammonia excretion. In metabolic acidosis, on the other hand, the decreased filtered load of bicarbonate diminishes the requirement of hydrogen ion for reabsorption of this moiety so that hydrogen ion secretion may actually fall with a negligible effect on net acid loss. The reduction in plasma CO_2 tension in this situation, though associated with a fall in the intracellular concentration of hydrogen ion, does not interfere with the regulatory response insofar as total acid-base balance is concerned. The reverse occurs in metabolic alkalosis, i.e., enhanced secretion of hydrogen ions, even though changes in plasma CO_2 tension may be less striking.

THE EFFECT OF THE INTRAVENOUS ADMINISTRATION OF POTASSIUM ON ACIDIFICATION

Berliner, Kennedy, and Orloff pointed out the significance of the reciprocal relationship between potassium and hydrogen ion secretion.[6] They suggested that these ions compete for some portion of the exchange system whereby sodium is reabsorbed. Figure 3 is from an experiment of Orloff and Davidson to illustrate certain features of this competition in the

chicken.[84] It should be noted that administration of potassium in one leg is followed by enhanced excretion of potassium and a rise in urine pH on that side. Presumably the entrance of potassium into tubule cells elevates cell pH, accounting for the observations. In both dog and man the infusion of potassium decreases the excretion of titratable acid and ammonia and increases that of potassium and bicarbonate. The secondary fall in urine pH

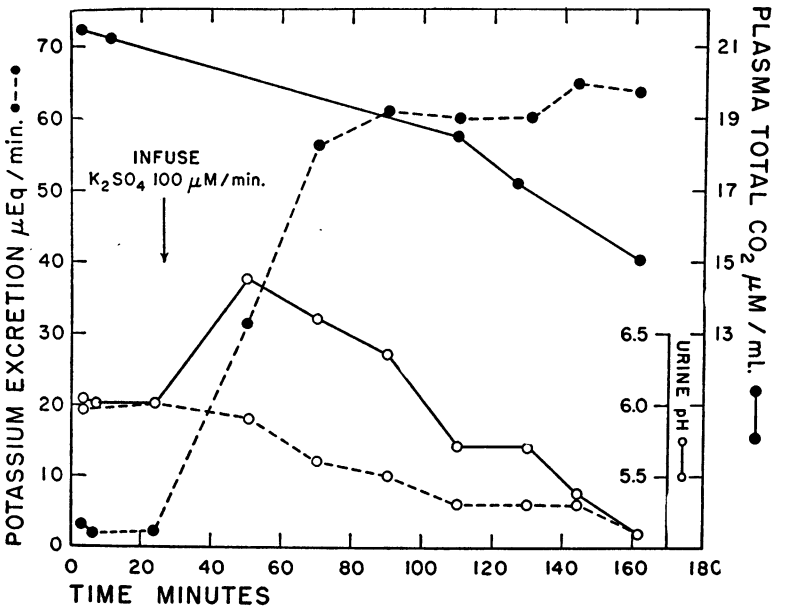

FIG. 3. The effect of intravenous administration of potassium on urine pH, potassium excretion, and plasma total CO_2 concentration. Urine potassium excretion is depicted for the experimental side only. Urine pH on that side is denoted by a solid line, on the control side by a broken line.

noted in the experiment shown in Figure 3 is undoubtedly related to the development of systemic acidosis, as evidenced by the fall in plasma bicarbonate. Acidosis is thought to be due to the extrusion of hydrogen ions from cells in exchange for entering potassium, although loss of alkali in the urine may contribute.[46] As noted earlier, a reduction in filtered load of bicarbonate diminishes the hydrogen ion requirement for bicarbonate reabsorption. Thus aciduria may supervene even when the over-all rate of hydrogen ion secretion is depressed. In the rat, on the other hand, potassium infusions uniformly produce acidosis and aciduria without the interposition of a period of urinary alkalinization.[25] Similar considerations apply to the development of so-called "resistance" to carbonic anhydrase inhibi-

tors. Administration of acetazoleamide, a potent carbonic anhydrase inhibitor, initially induces urinary alkalinization and kaluresis.[6] This may be dependent on an elevation of cell pH alone, though some fall in intracellular potassium concentration might be expected to occur after protracted use of the drug. "Resistance" occurs after prolonged administration as evidenced by aciduria despite metabolic acidosis.[28] This is not due to interference with the action of the drug, as has been suggested, but is related to the depression in filtered load of bicarbonate analogous to that observed after potassium administration. Since carbonic anhydrase is probably not involved in the hydrogen ion transport process, as noted earlier (Fig. 1) even were the enzyme completely inhibited, the uncatalyzed rate of hydration of CO_2 is sufficient for limited intracellular buffering and hydrogen extrusion.

HYPOKALEMIC ALKALOSIS

Potassium depletion is associated with extracellular alkalosis and intracellular acidosis secondary to the entrance of hydrogen and sodium ions into cells in exchange for potassium.[7, 12, 35] Aciduria and decreased potassium excretion noted in potassium-deficient animals is thought to be dependent on reciprocal changes in the hydrogen and potassium ion content of tubule cells. Though it has not been possible to confirm this by direct analyses, as in muscle, this is not surprising, since it would be unlikely that analyses of the whole organ could give valid information as to the chemical composition of the secretory cells.[12] In the light of these considerations, it is apparent that net acid loss from the body is not essential for the development of alkalosis in this situation as originally suggested. The function of the tubule cell is two-fold however: (a) it provides a route for potassium secretion during the development of hypokalemia (unnecessary when produced by gastrointestinal losses) and (b) it serves to perpetuate the alkalosis. Thus the maintenance of plasma bicarbonate at the elevated level induced by the transcellular exchanges indicates that hydrogen ion secretion is enhanced. The compensatory rise in plasma CO_2 tension may potentiate this response.

THE EFFECT OF SODIUM BICARBONATE ADMINISTRATION ON THE EXCRETION OF POTASSIUM

The infusion of sodium bicarbonate induces both kaluresis and alkalinization of the urine. Although superficially this appears to represent competition between hydrogen and potassium ions, intracellular alkalosis being the primary event, evidence in this regard is conflicting. Wallace and Hastings[57] noted no change in cell bicarbonate of muscle when the concentration of bicarbonate in the medium was altered, whereas Anderson and Mudge[1]

noted uptake of bicarbonate and potassium by kidney slices previously depleted of potassium. In contrast, elevation of medium bicarbonate concentration did not affect tissue bicarbonate significantly in slices in which the potassium content was normal. The infusion of sodium bicarbonate into one leg of the chicken does not produce unilateral alkalinization of the urine or kaluresis.[34] This is not necessarily at variance with the hypothesis that cell bicarbonate may rise under these circumstances, nor does it indicate some other cause for the reciprocal changes in electrolyte excretion. The addition of bicarbonate to blood elevates CO_2 tension acutely in the area drained by the vein. Were this sufficient (despite an increase in cell bicarbonate) to augment hydrogen ion secretion, any effect on urine pH and potassium excretion produced by the transfer of bicarbonate into cells might be masked. On the other hand, when bicarbonate is infused into a systemic vein of the chicken, even as in dog and man, kaluresis and alkalinization do occur. Since the rise in CO_2 tension under these circumstances is presumably less, due to the interposition of the lungs, it is possible that intracellular alkalosis may be implicated as the factor responsible for the urinary changes.

AMMONIA EXCRETION

In analyzing the mechanism of ammonia excretion a distinction between those factors which have to do with its intracellular production and those concerned with its transport from cells to urine is essential. In this regard the state of acid-base balance,[18, 44] the availability of precursor amino acids,[26, 33] the integrity of intracellular enzymatic processes[14, 25, 43] all are concerned with production, whereas urine pH,[9, 33, 49 58] urine flow,[33] and the permeability characteristics of the tubule cell membrane influence transport.[33] The concentration of ammonia within cells may be concerned with both processes.[33]

Ammonia is formed within renal tubule cells from precursor amino acids. Van Slyke considered glutamine the major source in the dog, but no confirmation of this observation in other species has been published.[56] Furthermore, little is known about the pathways of deamidation and/or deamination by which urinary ammonia is produced. That slices and homogenates of rat kidney deamidate glutamine to form ammonia is not necessarily proof that this is the major pathway for ammonia production in the intact animal. It is clear, for example, that in the normal dog other amino acids are capable of augmenting ammonia excretion markedly.[26, 33] Transamination may play an important rôle in this process.

The relationship between acid-base balance and ammonia excretion is well known.[18, 49] Thus the daily rate of ammonia excretion increases pro-

gressively in acidotic animals. This may occur in the absence of significant urine pH changes and may represent an increase in cellular production. The mechanism for this adaptive phenomenon has been studied extensively in other species. Davies and Yudkin[14] and others[25, 43, 44] noted increased glutaminase activity in homogenates and slices of renal cortex from acidotic rats. Whether other ammonia-producing enzymes adapt has not been conclusively established, nor has the stimulus to enzyme adaptation been defined. Leonard and Orloff[25] considered it to be related to acidosis since both they and Rector[43] noted in the rat that acetazoleamide-induced extracellular acidosis, despite urine alkalinization, is associated with enhanced ammonia excretion and increased deamidation of glutamine by slices and homogenates of kidney tissue. That this cannot be the only factor is indicated by the observation that hypokalemic alkalosis is also associated with glutaminase activation in this species.*[21]

It may be more reasonable to assume that factors related to intracellular pH and substrate concentration condition the adaptive response. Whether persistent aciduria and consequent enhanced ammonia excretion may under some circumstances stimulate cellular deamidative and deaminative processes is unknown. Leonard and Orloff noted an early rise in ammonia excretion in acidotic rats unassociated with a significant increment in glutaminase activity.[25] They interpreted this as indicative of the presence of other adaptive devices.

The inverse relationship between urine pH and ammonia excretion has been described by a number of investigators.[9, 33, 39, 58] The data are consonant with the view that NH_3, the free base, diffuses out of cells and accumulates in urine as NH_4^+, the extent of accumulation being a function of urine pH. The interposition of a perm-selective membrane freely permeable to NH_3 and less so or not at all to NH_4^+ is essential to this hypothesis.[20] The similar behavior of other weak electrolytes lends credence to this view.[33]

Recently Orloff and Berliner concluded that the accumulation of ammonia in urine reaches equilibrium only in the alkaline range of urine pH.[38] They have considered the kinetics of the process in the following manner. Ammonia diffuses out of cells into urine as a function of the permeability characteristic of the tubule membrane and the concentration of NH_3 in cell and urine respectively. At equilibrium, movement of ammonia in both directions is equal, and the concentration of NH_4^+ will be dependent on

* Acetazoleamide depresses ammonia excretion in dog and man in contrast to its effects in the rat. This throws some doubt on the view that glutaminase adaptation is a general phenomenon in all species.

urine pH.* In essence this is a restatement of the trapping hypothesis of Jacobs, Osterhout, and others who applied it to unicellular systems.[20, 22, 36] In view of the equilibrium nature of the process, it is not surprising that an increase in volume of the receiving fluid (urine) as occurs in diuresis should enhance ammonia excretion. In acid urines, on the other hand, ammonia excretion is independent of urine flow though inversely related to pH. Consequently, it is presumed that equilibrium is not achieved. Under these circumstances it has been postulated that movement of NH_3 is essentially unidirectional and that accumulation of NH_4^+ is limited by maximal rates of production and diffusion of NH_3 out of cells. The most acid urine accumulates the most ammonia by virtue of having been in the acid range over a longer segment of tubule, thereby receiving ammonia at a constant rate for a longer time. It is implicit in this hypothesis that neither acidification nor ammoniation are limited to an isolated segment of tubule, but occur over a variable length of tubule depending on buffer content and rate of hydrogen ion extrusion. In other terms, the most acid urine excreted has had hydrogen ions added to it more rapidly and over a greater length of tubule than any less acid urine of similar buffer content. Either mechanism for the accumulation of NH_4^+, the first a flow-limited equilibrium reaction and the second a production- and diffusion-limited process, is dependent on the differential permeability of the cell membrane to the uncharged and charged members of the buffer pair.

The substitution of ammonium ion for sodium in the urine in chronic acidosis serves to limit the loss of fixed cation from the body. This process was studied in detail by Gamble and by Sartorius.[18, 49] In order to effect the greatest conservation of base, urine pH must remain essentially constant or fall. Thus for each hydrogen ion neutralized by added ammonia, another must exchange for sodium or there will be no net gain of fixed cation by the body. That this does not occur under all circumstances is attested to by results of studies in which precursor amino acids were infused.[38] In these experiments urine pH invariably rose, presumably by virtue of titration with ammonia. Whether the sudden increase in intracellular ammonia production afforded by the added substrate elevates cell pH and interferes with acidification is unclear. Potassium excretion was unaltered, however, a situation not noted in other conditions in which cell pH is presumed to rise. If ammonium ion entered the urine by direct exchange with sodium rather than by diffusion of ammonia, this might conceivably result in interference

* This oversimplification of their analysis does not consider the effect on NH_4^+ accumulation of probable limited permeability of the membrane to NH_4^+; however, this is not crucial to an understanding of the process.

with both potassium and hydrogen secretion. Though there is no direct evidence to controvert this contention, the reproducible relationship between urine pH and ammonia excretion under other circumstances makes it unlikely. Futhermore, Lotspeich and Pitts did not observe urinary alkalinization when they infused amino acids into dogs.[26]

CONCLUSION

Though this was ostensibly a review of the renal regulation of acid-base balance, much has been omitted from consideration. This may be ascribed to ignorance as well as to limitations of space. Much of the discussion has been concerned with the theories of acidification and the factors determining the rate of hydrogen ion secretion. An analysis of base conservation and its rôle in acidosis and alkalosis has been avoided but is implicit throughout. Many of the concepts presented are controversial, the interpretations undoubtedly colored by the prejudices of the writer. Though it may not always be clear where fancy departs from fact, no apologies are offered. John Peters once wrote to the author: "The purpose of this—which I was constrained to publish—was to find out where we stood—and where to go from there. It was just an assemblage of question marks." This in essence was the avowed purpose of this effort.

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