THE EFFECT OF CARBON DIOXIDE ON THE KIDNEY

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PREVIOUS contributions to this symposium have analyzed the effects of carbon dioxide on the systems for the regulation of respiratory and cardiovascular function. The complexity of the mechanisms is by now obvious, as are the difficulties encountered in distinguishing primary and specific actions of CO_2 from the effects of the secondary concomitants of its actions. In this review, an attempt will be made to present in rather broad and general terms the renal mechanisms in which CO_2 plays an important role. Some overlap between material included in this review and in other papers in this symposium seems unavoidable, but it is hoped that the frame of reference may be sufficiently different that the repetition will be of merit. The specific effects of CO_2 on the neurohumoral mechanism, already discussed by Tenney, will be omitted entirely from this presentation. The bibliographies of the selected references cited should afford an opportunity for the interested reader to explore some of these problems more extensively.

RENAL HEMODYNAMICS

Systematic and well-controlled studies on the effects of CO_2 on renal perfusion, vascular resistances, glomerular filtration rate, etc. are not available. Observations on these parameters have been made during studies in which changes in CO_2 tension occurred, but so many other variables likely to alter hemodynamics were present that interpretation must be qualified with reservation.

Acute respiratory arrest in the harbor seal,[1] rabbit [2, 3] and dog [4-9] is known to result in a prompt and severe reduction in urine flow, associated, where measured, with a reduction in glomerular filtration rate (GFR), renal plasma flow (RPF), renal blood flow (RBF) and filtration fraction (F.F.-GFR/RPF). This response has been shown to depend in the rabbit and dog upon the integrity of the autonomic innervation of the kidney and can be significantly attenuated by surgical or pharmacological denervation of the renal pedicle. Studies during diffusion respiration have correlated oliguria and renal vasoconstriction with reduction in plasma volume, with autonomic vasoconstrictor impulses, with increased circulating catecholamines, with increase in cerebrospinal fluid pressure, and with hypercapnia and acidosis. The effect of diffusion respiration on mean arterial blood pressure has been variously reported and a direct relationship between RPF and mean arterial pressure noted. The severity of the oliguric response is reduced by concurrent administration of osmotic (mannitol) but not other (mercurials, xanthines, acetozolcamide) diuretics. Large doses of THAM (2-amino, 2-hydroxymethyl 1,3-propanediol), a compound which has properties of an osmotic diuretic and which also minimizes the hypercapnia, acidosis and increase in circulating catecholamines characteristic of diffusion respiration, likewise prevents oliguria during apnea. Inhalation of 30 per cent CO_2 results, in the lightly anaesthetized dog, in a profound fall in RBF and in urine flow, a response culminating in neurogenic renal vasoconstriction and severe oliguria.

On the other hand, hypercapnia induced in both dog and man by inhalation of CO_2 in moderate concentration has been associated with remarkably minor effects on RBF or GFR, particularly when anesthesia and surgical manipulation have been avoided and when hydration, plasma volume and ECF volume were maintained.[10, 21] In the chronic hypercapnia of pulmonary insufficiency, RBF and GFR do not appear to be systematically and consistently different from normal. Acute hypocapnia secondary to hyperventilation frequently produces a small reduction in GFR and in RPF [17, 22] and in salt depleted man was followed by a sharp depression in GFR, an effect abolished by the inclusion of CO_2 in the gas mixture inhaled during hyperventilation.[23]

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TABLE 1

APPROXIMATE IONIC COMPOSITION OF BODY FLUIDS

	Plasma	Interstitial	Intracellular
Na^+ mEq./l.	140	144	10
K^+ mEq./l.	5	5	165
Ca^{++} mEq./l.	5	2.5	30
Mg^{++} mEq./l.	3	1.5	
Cl^- mEq./l.	103	115	
HCO_3^- mEq./l.	27	30	
$H_2PO_4^-/HPO_4^=$ mEq./l.	2	2	
$SO_4^=$ mEq./l.	1	1	
Other mEq./l.	5	5	150
Protein mEq./l.	15	0	55
*p*H	7.40	7.45	6.1–7.1
P_{CO_2} (mm. Hg)	45	45	45

RENAL ELECTROLYTE EXCRETION

The most extensively studied effects of CO_2 on the kidney concern its role in the renal mechanisms involved in the regulation of the ionic composition and *p*H of body fluids. Average normal values for the major ionic constituents of plasma and an ultrafiltrate thereof, interstitial fluid are shown in table 1. The final column is a rough average of various estimates of the ionic composition of muscle cells, and presumably reflects the chemical anatomy of cells in general. Cells contain little sodium and potassium is the predominant cation; moreover, the anions are very different from those of ECF, being largely proteinates and organic phosphates, a large fraction of which do not freely cross the cellular membrane. The question of why the chemical anatomy shown in table 1 should be characteristic for modern vertebrates is interesting. Extracellular fluid resembles closely the composition believed to have been prevalent in the Precambrian oceans from whence the ancestors of modern vertebrates migrated to the continental land masses. Those forms which later returned to the oceans have evolved with the development of a great variety of mechanisms to cope with the progressively increasing salinity of the seas. However, natural selection operating over half a billion years seems to have stabilized the composition of the ECF of terrestrial vertebrates in a pattern approximating that which the first migrants must have brought with them from their previous marine environment. Given this composition, we shall be concerned with the pulmonary and renal mechanisms which maintain it.

As a preamble to the discussion, some fundamental relations may profitably be reviewed. *p*H refers to the negative logarithm of the $[H^+]$. An acid is defined as a hydrogen ion (proton) donor, a base, a hydrogen ion acceptor; one increases, the other decreases, the free $[H^+]$. Thus hydrochloric, lactic, and citric acids all contribute hydrogen ions to solution. The associated anions, chloride, lactate and citrate are not acids. Lactate and citrate are bases, in the sense that they associate with protons to yield acids of limited dissociability. Carbon dioxide is not an acid *per se*, but in solution, enters into reactions that are important in regulating the acidity of body fluids (table 2, reaction 1). Except under unusual circumstances, *e.g.*, exhibition of carbonic anhydrase inhibitors, and even then, only at sites where CO_2 is either being rapidly introduced into or evolved from body fluids, these

TABLE 2

SOME REACTIONS IMPORTANT IN BUFFERING BODY FLUIDS

1. $CO_2 + H_2O \underset{\text{Anhydrase}}{\overset{\text{Carbonic}}{\rightleftharpoons}} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$

2. $CO_2 + OH^- \underset{\text{Anhydrase}}{\overset{\text{Carbonic}}{\rightleftharpoons}} HCO_3^-$

3. $RCOO^- + H^+ \rightleftharpoons RCOOH$

4. $RNH_2 + H^+ \rightleftharpoons RNH_3^+$

5. $$pH = pK_s + \log \frac{[\text{Proton Acceptor}]}{[\text{Proton Donor}]}$$
$$= pK_{HA} + \log \frac{[A^-]}{[HA]}$$
$$= pK_{RNH_2} + \log \frac{[RNH_2]}{[RNH_3^+]}$$
$$= pK_{RCOOH} + \log \frac{[RCOO^+]}{[RCOOH]}$$
$$= pK'_{H_2CO_3} + \log \frac{[HCO_3^+]}{[H_2CO_3] + [CO_2]}$$
$$= pK'_{H_2CO_3} + \log \frac{[HCO_3^-]}{\alpha p CO_2}$$

reactions may be assumed to be at equilibrium. An increase in [CO_2] drives reaction 1 rightward with the production of free hydrogen ions from carbonic acid. HCO_3^-, which can react with free hydrogen ions and thus reduce their concentration, is a base. An increase in [HCO_3^-] drives reaction 1 leftward, decreasing [H^+] and drives reaction 2 in the same direction, increasing [OH^-]. These two reactions are operationally identical since [OH^-] [H^+] = Kw = 10^{-14}. The complete oxidation of many organic anions generates HCO_3^- ions. It is the lactate ion which is oxidized thus: $C_3H_5O_3^- + 3O_2 = 2CO_2 + 2H_2O + HCO_3^-$, that is the base, not the cation, *e.g.*, sodium with which it may have been associated or administered. Such acids as lactic and citric contribute free hydrogen ions but in time, they are completely oxidizable to CO_2 and water ($C_3H_6O_3 + 3O_2 = 3\ CO_2 + 3\ H_2O$) so that in the final analysis, they have an acidifying potential equivalent to the CO_2 derivable from their oxidation. In the ECF, the $CO_2/H_2CO_3/HCO_3^-$ system is the major source or sink, *i.e.*, buffer system, for free hydrogen ions. In cells, however, [HCO_3^-] is much lower than in ECF, but other proton acceptor groups are present in the various proteins and organic phosphorus compounds. In cells, reactions 3 and 4 of table 2 take place, and are supplementary to as well as in equilibrium with reactions 1 and 2. Hydrogen ions gain access to cells via CO_2 diffusion across the membrane and subsequent movement of reaction 1 rightward; the hydrogen ions produced titrate cellular buffers by reactions 3 and 4. There is also evidence that direct H^+ exchange for Na^+ and K^+ may occur, and more recently that basic amino acids such as lysine may transport hydrogen ions in the form RNH_3^+ in exchange for cellular cation.[24] In some of these mechanisms, changes in cellular osmolality occur, necessitating osmotic equilibration of ICF with ECF. This may be accomplished by water movement in one direction or ionic movement in the opposite direction. The *p*H at any locus in the body may be defined in terms of the dissociation constant and the relative concentrations of dissociated and undissociated species of any or all of the buffer pairs present, (reaction 5, table 2). In ECF, the most easily measured is the CO_2 system. Cellular *p*H is not amenable to easy measurement and various methods yield results which are not entirely concordant.[25, 26] Cellular CO_2 tension can be assumed to equal that of the plasma, but the determination of cellular [HCO_3^-] presents serious difficulties. However, as will appear, much of the current theory about acid-base balance depends on inference to this datum of cell *p*H.

The Problem of Acid Base Homeostasis in the Normal

The normal adult human is, over any reasonable period of time in balance, *i.e.*, the organism neither gains nor loses but is constant with respect to the water and ionic composition of tissues. This means that the total output is equal to the total intake. Dietary protein, carbohydrate and fat enter metabolic pools, become incorporated into cellular structure, are oxidized to provide energy, and products of their oxidation are excreted. Whether the excreted output is endogenous or exogenous in origin is by and large immaterial; what is essential is that it is equal to the exogenous intake. Oxidative metabolism produces CO_2 at a rate of about 25 m*M*/minute, carbonic acid is formed from it, the hydrogen ion thereby generated is buffered, and the CO_2 content of blood is increased. In the lungs, reversal of this reaction leads to CO_2 excretion. Any disparity between the rates of production and excretion of CO_2 will result in an accumulation or deficit in volatile acid, and will be reflected in a departure of arterial *p*H and P_{CO_2} from normal. Both of these constitute stimuli which appropriately depress or accentuate alveolar ventilation in the direction required to restore balance. Metabolic oxidations also yield H_2SO_4 and H_3PO_4 from sulfur-containing amino acids, from nucleoprotein phosphorus and from phospholipid phosphorus. These are nonvolatile acids, and homeostasis requires that both the H^+ as well as the associated anion be excreted at rates essentially equivalent to the rates at which they are produced. The acids are first buffered, transported to the kidney and finally excreted; disparity in the two rates leads to accumulation or depletion of fixed acid. It is apparent that the order of magnitude of fixed acid production, estimated to be about 0.05 mEq./minute, is trivial compared to that of volatile acid, and that a relatively

small alteration in the rate of dissipation of volatile acid thru the lungs might easily vitiate the impact on *p*H of a disparity between the rates of production and excretion of fixed acid. Such respiratory compensation, however, is achieved at the expense of an altered arterial P_{CO_2}, which in turn, constitutes an important signal to the kidney to modify fixed acid excretion appropriately.

Acidosis and Alkalosis

Four situations are classically categorized in which abnormal requirements are imposed on this homeostatic mechanism. (1) Metabolic acidosis occurs whenever the endogenous or exogenous load of non-volatile acid exceeds renal excretory capacity. (*e.g.*, diabetic acidosis or ammonium chloride ingestion) or excessive loss of alkali occurs (infantile diarrhea). (2) Metabolic alkalosis is due to abnormal loads of alkali (excessive intake of $NaHCO_3$) or abnormal losses of acid (HCl in vomitus). A special case of the latter involves simultaneous potassium depletion. The decrease in the *p*H of arterial blood in metabolic acidosis provides the stimulus for increasing alveolar ventilation, and thus increased dissipation of volatile acid compensates, at least partially, for increased accumulation of fixed acid. Compensation by volatile acid retention to minimize the impact of metabolic alkalosis is also demonstrable under some circumstances but quantitatively is not so effective.[27, 28] It appears that reduction of alveolar ventilation to the extent required to effect compensation is limited and that the respiratory control under these circumstances is more influenced by P_{CO_2} and P_{O_2} than by *p*H. The acute respiratory adjustments are stopgap measures and restoration of the *status quo ante* depends on appropriate renal disposition of the nonvolatile acid or alkali. (Obviously net electrolyte and water losses, *e.g.*, in vomiting and diarrhea, must be replaced from external sources.) (3) Respiratory acidosis represents a failure of pulmonary CO_2 excretion to occur at a rate equivalent to CO_2 production without elevation of arterial P_{CO_2} and $[HCO_3^-]$ and a fall in arterial *p*H, the necessity for the elevation of CO_2 tension resulting from inadequate ventilation or from CO_2 inhalation. (4) Respiratory alkalosis represents a state in which pulmonary CO_2 balance is attained at a lower than normal arterial P_{CO_2} because of alveolar hyperventilation; arterial P_{CO_2} and $[HCO_3^-]$ are reduced and *p*H increased. The signal to the kidney is mediated to large extent through the arterial CO_2 tension, and the mechanisms responsible for appropriate changes in acid excretion are initiated by this stimulus. Thus primary respiratory dislocations are compensated by countervailing renal changes directed at restoring arterial *p*H to normal in spite of the pulmonary abnormality. Renal adjustments operate rather slowly.

The bulk of our present understanding of these processes stems from studies on experimentally induced respiratory or metabolic acidosis and alkalosis. Two aspects of the problem may be considered. One concerns the extrarenal adjustments to the challenge; the other, the renal reactions.

Extrarenal Effects of Acidosis and Alkalosis

The renal response to acidosis or alkalosis is largely conditioned by the alterations in ionic composition produced both in the tubular cells and in the blood and interstitial fluid perfusing them. It would seem worthwhile then to examine the initial dilution and distribution of acid or alkaline loads added to the ECF in the absence of kidneys, plotting, as it were, a titration curve of the whole organism as the various buffers within and without the ECF interact with the load. Such an examination must perforce include evaluation of the direction and magnitude of the changes in volatile acid excretion thru the lung which occur as part of the response. The steady state which obtains upon completion of the extrarenal adjustments furnishes the backdrop against and because of which renal responses occur. In the nephrectomized animal, as well as in the intact animal and man whose simultaneous renal adjustments are given appropriate consideration, a fairly satisfactory description of these extrarenal adjustments has become available.[13, 29-33] There remain, however, a number of points on which various studies are not concordant, and the precise localization and quantification of intracellular fluid compartment changes to specific tissues still leaves much to be desired.

Metabolic Acidosis. In acute experimental

acidosis induced by HCl administration to the dog, the bulk of the acid anion, Cl^-, may be accounted for in the ECF and in erythrocytes. At least 40 per cent of the H^+ load reacts with ECF HCO_3^- and 10 per cent with erythrocyte HCO_3^-; the CO_2 so generated eventually being transported to and dissipated through the lungs. The remainder escapes from ECF, and concomitantly an approximately equal amount of cation, predominantly Na^+ but also K^+, is transferred into the ECF. The extent to which this exchange is with structural tissues such as bone,[24] or with the ICF is not completely clear. The largest component of ICF is in skeletal muscle, and the intracellular *p*H of muscle has been found only slightly, if at all, reduced in metabolic acidosis. The large loss of volatile acid through the lungs blunts the impact of the fixed acid load very considerably, but not completely. In so doing the total $[CO_2]$ of ECF is reduced, with the fractional reduction in $[HCO_3^-]$ exceeding that in P_{CO_2}. Metabolic acidosis occasioned by a surfeit of endogenous acid production or by alkali loss is characterized by changes that are qualitatively similar.

Metabolic Alkalosis. The induction of metabolic alkalosis by the acute administration of $NaHCO_3$ results in the retention of 70–80 per cent of the infused cation in the ECF, the remainder gaining access to the intracellular compartment in exchange for H^+ derived from proton donor groups in tissue buffers. The relative roles of bone *vis à vis* intracellular fluid are qualitatively undefined. A fraction of the infused HCO_3^- reacts with H^+ from bone, cells, lactic acid (whose production seems to be increased by a direct effect of alkalosis on intermediary carbohydrate metabolism[35]) and proton donors in ECF to form H_2CO_3. In man, at least, during the infusion of $NaHCO_3$, there is a considerable pulmonary loss of this H_2CO_3, accentuating the alkalosis by volatile acid depletion. Pulmonary CO_2 retention supervenes after discontinuation of the infusion and it would appear that a large fraction if not all of the HCO_3^- not neutralized by lactic acid remains in the body, localized, by and large in ECF. Plasma P_{CO_2}, $[HCO_3^-]$ and [lactate] are elevated. Muscle cell *p*H is normal or slightly elevated. The magnitude and direction of K^+ transfers has been variously described, but acutely they appear to be relatively unimportant. In chronic metabolic alkalosis K^+ depletion is frequent and both here as well as in the metabolic alkalosis that develops as a result of primary depletion of K^+, reduction in ICF $[K^+]$ is well established.[36, 37]

Acute Respiratory Acidosis. The induction of acute respiratory acidosis experimentally by CO_2 inhalation results in the accumulation of volatile acid until the arterial CO_2 tension increases to the point at which the A-a CO_2 gradient and the alveolar ventilation are adequate for the reestablishment of CO_2 balance. The magnitude of this accumulation is difficult to quantify with precision but it is generally believed that the bulk of the HCO_3^- formed when reaction 1 is driven to the right remains in ECF; Na^+ and K^+ from bone and ICF exchange for ECF H^+, matching about 50 per cent of the ECF HCO_3^- increment. Cl^- transfer to erythrocytes "frees" ECF cation to match another 30 per cent of the HCO_3^- increment, a transfer made possible by the change in erythrocyte buffers (increase in cation and disappearance of anion) by reactions 3 and 4 (table 2). In addition, plasma protein titration, decrease in anaerobic glycolysis and lactic acid production, transfer from ICF to ECF and titration of inorganic phosphate buffer account for the remainder of the buffering of the increment in volatile acid. The new steady state is characterized by reduction in arterial *p*H, a fractionally greater elevation of arterial P_{CO_2} than $[HCO_3^-]$ and presumably a high cellular P_{CO_2}. Skeletal muscle *p*H is unequivocally acid. In *chronic* respiratory acidosis, the participation of the erythrocytes in the buffering process seems to be of a lesser magnitude than that observed acutely in the nephrectomized animal, and chloride diuresis of substantial magnitude occurs when the kidney is present.[38–40]

Respiratory Alkalosis. The induction of respiratory alkalosis by hyperventilation results in an increased pulmonary dissipation of volatile acid until the new A-a gradient and alveolar ventilation reach levels at which balance is restored. Again the magnitude of the acid loss has not been precisely evaluated in data currently available in the literature. Reaction 1 is shifted to the left, and ECF $[HCO_3^-]$ is reduced by its conversion to H_2CO_3 thru interaction with H^+ derived from proton donors in bone and ICF, made available by

the movement of Na^+ and to a lesser extent K^+ into the sites whence H^+ was derived. Titration of erythrocyte buffers in the direction opposite to that occurring in respiratory acidosis makes possible the movement of Cl^- from red cells into ECF. Lactic acid production is accentuated and makes H^+ available for reaction with HCO_3^-, lactate appearing in ECF. After the initial depletion of H_2CO_3 from ECF HCO_3^- by these mechanisms, a steady state, characterized by an increased arterial *p*H and a reduced arterial $[CO_2]$ fractionally greater for P_{CO_2} than for HCO_3^- is observed. Measurements of *p*H of skeletal muscle show a rise in *p*H. The relative contribution of H^+ from bone *vis à vis* other phases of ICF is not well defined.

Renal Effects of Acidosis and Alkalosis

The renal response to dislocations in body fluid ionic composition is well known in the general teleological sense that it operates to negate the abnormality present. Partition by the kidney of the ECF reaching it into two components, an acid urine and an alkaline reabsorbate will rid the organism of acid and back titrate body fluids toward alkalinity. Conversely, the formation of a urine more alkaline and a reabsorbate more acid than ECF in the alkalotic state will result in restoration of body fluid composition toward normal *p*H. The demands on the renal mechanism, however, are not limited to the excretion or retention of H^+ but require integration of the mechanisms concerned with H^+ excretion and appropriate mechanisms for the retention or rejection of base (HCO_3^-), cation (Na^+), and anion (Cl^-, SO_4^-, $H_2PO_4^-/HPO_4^=$). Certain gross features and their significance should be noted. An acid urine, whose maximal *p*H in man is about 4.5–5.0 is excreted during acidosis. The fact that a hydrogen ion gradient of 1,000 to 1 between urine and plasma (urine *p*H 4.4, plasma 7.4) appears to limit renal acidification has not been explained on any theoretical basis, but is an empirical observation that is valid for the normal kidney and for most varieties of renal disease. In renal tubular acidosis, however, maintenance of a gradient higher than 50 to 1 is not possible.[11, 12] Even acidification to the maximal limit does not, *per se*, represent the dissipation of much hydrogen ion. A liter of *p*H 4.0 urine contains only 0.1 m*M* of free hydrogen ion, an amount that is trivial in comparison to the need of a patient in diabetic acidosis, for instance, to dispose of acid at the rate of 500–1,000 m*M*/day. However, since urine may contain hydrogen ion bound to buffers, such as $H_2PO_4^-$, the total hydrogen ion content can be greater than that represented by free H^+. The excretion of H^+ bound to buffer is called titratable acid. This is estimated by the amount of OH^- necessary to titrate the urine back to plasma *p*H, the inverse of the amount of acid added to bring it to urine *p*H. Titratable acid excretion may reach a rate as high as 0.5 m*M*/minute under appropriate stimulation. H^+ is also present in the urine as NH_4^+. Ammonia is formed in tubular cells from amino acids, principally glutamine. In some species, the enzyme glutaminase which catalyses the formation of ammonia from glutamine is adaptive, its concentration increasing rapidly in acidosis. The amount of ammonia found in urine is related directly to the *p*H of the urine; the more acid the urine, the more ammonia is present. The explanation advanced for this is as follows: The tubular epithelium is more permeable to NH_3 than to NH_4^+. Ammonia formed intracellularly tends to diffuse outward in all directions along its concentration gradient. If the urine is acid, ammonia reaching the urine reacts with H^+ to form NH_4^+. Thus the free $[NH_3]$ remains low in urine, and the concentration gradient remains more favorable for diffusion into urine than into any other compartment. Ammonium ion excretion may reach values of 0.5 m*M*/minute under maximum stimulation. The addition of NH_3 to urine with the formation of NH_4^+ also provides cation and reduces the amount of "fixed" cation (Na^+ and K^+) needed to match non-volatile acid anion (Cl^-, $SO_4^=$, $H_2PO_4^-/HPO_4^=/_2$ hydroxybutyrate) requiring excretion. An acid urine contains very little HCO_3^-, so that the process of acidification results in the complete or almost complete salvage of the filtered HCO_3^-. Thus in metabolic acidosis the excess hydrogen ions are excreted on buffers and on ammonia in the process of which anion may be excreted without "fixed" cation; reabsorption of HCO_3^- with it cation is equalent to retention of alkali.

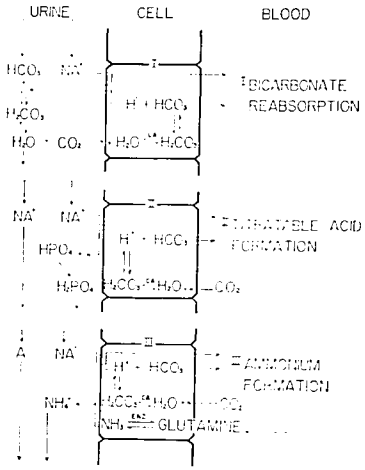

FIG. 1. Mechanisms for urinary acidification by ionic exchange between urinary Na^+ and tubular H^+.

The excretion of an acid urine thus rids the organism of the offending acid and anion, and simultaneously operates in the direction of restoring the bicarbonate lost as volatile acid thru the lungs in the initial respiratory compensation. In respiratory acidosis, the excretion of an acid urine again salvages HCO_3^-, and, by excretion of fixed acid at a rate higher than that at which it is formed, permits Na^+ and HCO_3^- to accumulate to the point at which a normal *p*H is more nearly approached despite the respiratory disability.

In alkalosis, the picture is reversed. The need of the organism is to salvage acid or otherwise stated, to excrete alkali. Again, there is an empirically observed H^+ gradient limit of about 1 to 6 from urine to plasma, since urine more alkaline than *p*H 8.2 is seldom observed. If the urine is alkaline, the reabsorbate must be acid to the arterial perfusate and the acidified venous effluent titrates body fluids back toward normal. In both metabolic and respiratory alkalosis, the over-all operation is excretion of Na^+ and HCO_3^-, the loss of which in each case tends to titrate body fluids back toward normal.

RENAL MECHANISMS INVOLVED IN URINARY ACIDIFICATION [13, 45-49]

Early speculation about the nature of the acidification process sought to distinguish between H^+ addition to urine or base (*e.g.* $HPO_4^=$ and HCO_3^-) reabsorption from urine. Either process increases urinary acidity. During the period 1944–1950, Pitts reported a brilliant series of studies in dog and man from which he argued that acidification could not be accounted for by the reabsorption of base from glomerular filtrate, unless the possibility of $CO_3^=$ reabsorption be entertained. This latter suggestion is considered unlikely since the concentration of $CO_3^=$ in body fluids and urine is vanishingly small. Another question posed was whether H^+ addition occurred by ionic exchange of H^+ from cells with Na^+ from urine, or by addition of "molecular" acid, *e.g.*, by the addition of CO_2 or $H_2PO_4^-$. This question was resolved by Pitts in favor of ionic exchange. The theory of hydrogen ion addition by ionic exchange allows for a comprehensive and simple explanation for acidification. As shown in figure 1, the exchange process includes a mechanism for acidification, and also for the simultaneous changes in cation reabsorption which are associated with HCO_3^- reabsorption and the excretion of titratable acid and ammonium. The source of the extruded H^+ shown in this figure is from CO_2 thru H_2CO_3. Whether or not this or some other metabolic reaction generates the H^+, its extrusion reduces cell $[H^+]$ with a rise in cellular $[OH^-]$. It has been suggested that CO_2 might buffer OH^- and that the role of carbonic anhydrase is to catalyse the latter reaction (table 2, reaction 2). From the over-all point of view, either reaction yields HCO_3^-, and at least in the formation of titratable acid and NH_4^+, requires that the CO_2 utilized be replaced from plasma. The rate of extrusion of H^+ is dependent, among other things, upon the cell $[H^+]$, cellular acidosis increasing this rate, cellular alkalosis reducing it. Cellular *p*H in turn is related to cellular and ultimately to arterial plasma P_{CO_2}. The change in cellular *p*H with change in arterial CO_2 tension depends on the change in cellular $[HCO_3^-]$. From the previous discussion of the changes

in the *p*H of muscle cells, it would seem reasonable to presume that the *p*H of renal tubular cells is reduced in respiratory acidosis and increased in respiratory alkalosis; in metabolic disturbances, the changes are probably slight, but in the same direction. In respiratory acidosis, therefore, the high cellular $[H^+]$ results in rapid H^+ dissipation thru the kidneys by reabsorption of HCO_3^- and excretion of TA and NH_4^+, all three mechanisms resulting in retention of fixed cation. It should be noted that the reduced filtered load of HCO_3^- in metabolic acidosis reduces the magnitude of H^+ extrusion required to render the urine HCO_3^- free, and permits the diversion of more H^+ to TA and NH_4^+. Marked acidification need not imply a high cell $[H^+]$. In respiratory alkalosis, the low cellular $[H^+]$ provides a basis for reduction in its rate of extrusion, to an extent such that even the low filtered HCO_3^- load exceeds the rate of H^+ extrusion and HCO_3^- escapes reabsorption. The high filtered load of HCO_3^- of metabolic alkalosis might be expected to eventuate in incomplete HCO_3^- reabsorption, and consequently in the excretion of an alkaline urine even if tubular cell $[H^+]$ were normal. Changes in the *p*H and/or P_{CO_2} of tubular cells on the reabsorption and excretion of HCO_3^- cannot be viewed apart from the effect of the stimulus producing them on the extrarenal buffering and pulmonary excretory processes which independently modify plasma $[HCO_3^-]$ and thereby filtered HCO_3^- load.

Quantitatively, HCO_3^- is the most important proton acceptor present in glomerular filtrate due to its high concentration and *p*Ka, and until most of it has been reabsorbed, urine *p*H is not substantially reduced. The low concentration and, for many, the lower *p*Ka of other urinary buffers preclude the formation of much TA and significant NH_4^+ accumulation in a nonacidified urine. Nevertheless, while acidification proceeds at whatever rate, water and NaCl reabsorption occur continuously, and the concentrations of HCO_3^-/H_2CO_3/CO_2 tend to increase. Since CO_2 can escape to blood thru a permeable epithelium and since its rising urinary concentration establishes a favorable gradient for its diffusion into blood, actual acid loss occurs from the other proton donors in urine, as outward diffusion of CO_2 shifts to the right these reactions:

$$\overset{Na^+}{H_2PO_4^-} \rightleftharpoons HPO_4^- + H^+$$

$$\overset{Na^+}{H^+ + HCO_3^-} \rightleftharpoons H_2CO_3 \rightleftharpoons H_2O + CO_2\uparrow$$

The acid returned to blood in this process opposes the effect of the acid added to urine in the course of the H^+ for Na^+ exchange reaction. Both processes obviously operate concomitantly, and in a sense, early bicarbonate reabsorption must be attenuated to some degree if the alkalinization by water reabsorption is to be effective further along the tubule in alkalinizing urine.

Relationship between Acidification and Potassium Excretion [36, 37, 45-48]

The point of view that acidification is determined by cell *p*H has recently had to be modified by the evolution of our understanding of mechanisms involved in K^+ excretion. It is now well established that K^+ may, under appropriate circumstances, be excreted in amounts greater than that available from glomerular filtrate. This recognition of the possibility of K^+ secretion has rendered somewhat ambiguous the interpretation of changes in K^+ excretion, since it is clear that this ion may and usually is reabsorbed almost completely from glomerular filtrate, while tubular extrusion may also occur. An increase in K^+ excretion may mean less reabsorption or more secretion. Conversely, a fall in K^+ excretion may be interpreted as a decrease in secretion or an increase in reabsorption.

The hypothesis that complete K^+ reabsorption occurred proximally, and that the regulation of K^+ excretion was thru a distal secretory mechanism has been extremely useful for interpreting a wide variety of experimental data. The support for this hypothesis has been reviewed by Berliner.[36] Unfortunately recent micropuncture data are not consonant [50, 51] and the simplifying assumption of complete proximal reabsorption may have to be revised. The location of the secretory mechanism in any case seems clearly to be a relatively distal one, and must, in effect, involve ionic exchange, K^+ moving from cell to urine while Na^+ moves in the opposite direction.[52] It

might be noted that K^+ secretion is dependent on the availability of counter-ion in urine; when Na^+ reabsorption is excessive proximal to the site of this mechanism, the reduced load of Na^+ becomes a limiting factor in the secretion of K^+.

A number of relationships between K^+ excretion and acidification have long been known, and the proposal [53] that the K^+ secretory mechanism was also responsible for H^+ secretion, with the two ions competing for sites on a common carrier molecule in proportion to their intracellular concentrations has been useful in explaining many features of electrolyte excretion. Obviously, assumptions become compounded in some of these arguments. The difficulty in assessing cell *p*H has already been pointed out. Difficulties also attend the evaluation of cell $[K^+]$. In general, however, it has been shown for many experimental and clinical situations that acidification and K^+ excretion are inversely related to each other, acidification predominating when cell $[H^+]$ or cell $[H^+]/[K^+]$ is increased, while alkalinization predominates when cell $[H^+]$ or $[H^+]$ $[K^+]$ is reduced. The rate of excretion of each seems to be regulated by their individual cellular concentrations, as well as by the relative concentrations of the one with respect to the other. Such an H^+ and K^+ for Na^+ exchange process can perform all of the operations shown in figure 1 when H^+ extrusion predominates, and one might ask whether it is the sole mechanism of acidification. This question was more relevant before the recent review and revision of sites of localization within the nephron of acidification.[54, 55] Proximal as well as distal acidification has now been clearly demonstrated in the rat nephron, and further acidification in the collecting ducts of hamsters also shown. Since it is highly unlikely that K^+ secretion occurs proximally, the acidification mechanism operating there must be distinct from the K^+-linked distal mechanism; transport of K^+ does not seem to occur in the acidification site in collecting ducts. The data presently available then would seem to indicate that H^+ for Na^+ exchange operates in all areas of the nephron but is coupled competitively with a K^+ secretory mechanism in the distal tubule.

Reabsorption of Bicarbonate

The reabsorption of HCO_3^- with its accompanying cation is an important feature of acidification and as such has been subjected to a tremendous amount of study in the last 10 years.[11-22, 54, 56, 57] Recent data indicate that at least 80 per cent and perhaps as much as 90 per cent of the bicarbonate is reabsorbed proximally; [54] the remainder, plus any added in the loop of Henle, is reabsorbed at more distal sites. As has been emphasized, a coherent explanation for it can be offered in terms of mechanism *1* of figure 1. However, a categorization of some of its characteristics has led several investigators to a bias in favor of ionic bicarbonate reabsorption. If the filtered load of bicarbonate is progressively increased from low to high values, reabsorption is essentially complete until a more or less well defined "threshold" value is reached; thereafter, rate of reabsorption becomes fixed, the remainder of the filtered load appearing in the urine. This resembles the "titration" curves described for substances such as glucose, and interpreted as indicating an active reabsorptive mechanism, in which step two in the reaction between substrate and enzyme or carrier, $S + E \rightleftharpoons ES \rightarrow E + P$ is rate limiting.[58] Accordingly, the first step reaches equilibrium as [S] increases and further urinary S can be coupled to carrier only to the extent that E is uncoupled from the product. Thus the carrier becomes saturated and a maximal rate of transport is attained. The maximal rate of bicarbonate reabsorption was subsequently discovered to be directly and linearly related to arterial CO_2 tension; however, at any constant value of CO_2 tension, the same type of relationship between reabsorption and load was apparent. Under certain circumstances, HCO_3^- seemed to compete with Cl^- for reabsorption. Moreover its reabsorption could be specifically inhibited by a number of carbonic anhydrase inhibitors, the inhibition at any given dosage level being, according to at least one group of investigators, of a constant amount, regardless of P_{CO_2}. Finally, from an analysis of the rate of HCO_3^- reabsorption at a variety of dosage levels of acetazoleamide, it appeared that HCO_3^- reabsorption could be described in terms of a

kinetic analysis in which carbonic anhydrase served as enzyme and HCO_3^- as substrate. A critique of the merits of the two theses, H^+ exchange for Na^+, etc., *vs* ionic HCO_3^- transport is beyond the scope of this essay. It seems probably that either are compatible with most of the observations, and certain conceptual difficulties in defining the detailed mechanism for ionic HCO_3^- transport with carbonic anhydrase as catalyst render the hypothesis less convincing than the ionic exchange mechanism in the opinion of this reviewer.

Carbon Dioxide Tension of Urine

A fairly large body of data, recently summarized,[59] has accrued in relation to the problem of explaining the disparities observed between urine and plasma CO_2 tension. Throughout the previous discussion, it has been implied that the renal tubular epithelium is freely permeable to CO_2 and that urine and plasma are at essentially the same CO_2 tension. While differences are usually small, certain situations are characterized by large disparities. Urine CO_2 tension tends to be higher than that of plasma when the urine is alkaline and the increment tends to be directly related to the concentration of nonbicarbonate buffer present. Carbon dioxide tension lower in urine than in plasma tends to be found when the urine is poorly buffered and fairly acid. Acetazoleamide elevates urinary CO_2 tension above that of plasma in poorly buffered urine but its effect is minimal in well buffered urine. Lactic acid loading frequently reduces urinary below plasma CO_2 tension.[60]

While the mechanisms responsible for these observations are not subject to a concensus, some aspects seem clear. The tubular epithelium cannot be uniformly permeable to CO_2, else such gradients could not occur. Whether the impermeability is a membrane characteristic or related to geometry (surface area/volume) and to flow rate is not clear, but most investigators assign considerable importance to the latter factors. Finally, the rates at which reaction 1 of table 2 occur are important. If bicarbonate is converted by H^+/Na^+ exchange to H_2CO_3, and urine is excreted before the carbonic acid dehydrates to CO_2 and water, a urinary CO_2 tension higher than that of plasma will occur. This was the mechanism first invoked by Pitts to explain the phenomenon of high urinary CO_2 tensions and was supported by the subsequent demonstration that intravenously administered carbonic anhydrase gained access to the urine, and that its appearance therein was associated with the disappearance of CO_2 tension gradients. Such observations were interpreted to indicate that in the absence of catalysis some of the carbonic acid flowed into a CO_2 impermeable segment, and CO_2 subsequently formed from it could no longer escape. In the presence of a catalyst, dehydration of the carbonic acid to CO_2 was accelerated and the urinary CO_2 equilibrated with that in the tubular perfusate before flowing into the impermeable segment. *In vitro*, however, it has been demonstrated in a model system in which the CO_2 tension is maintained as nearly constant as possible that the dehydration reaction under discussion reaches equilibrium extremely rapidly even in the absence of catalysis unless the concentration of nonbicarbonate buffer is significant (5 m*M*/L). It is difficult to reconcile this observation with the fact that high urinary CO_2 tensions may be observed in poorly buffered urine, unless it is assumed that the H_2CO_3 formation responsible for the high tensions occurs very close to the end of the nephron (collecting duct.) If this were the case, it is difficult to understand why catalysis in the urine should so effectively abolish the gradients. The collecting duct differs from the other parts of the nephron in that it has a surface area that is small relative to volume and volume flow, and in that its larger diameter implies longer distances over which diffusion processes must occur. Acidification and ammoniation are now known to occur in this locus. The former may elevate CO_2 tension, the latter depress it. The operation of these processes in the preterminal locus described may be the principal determinants of urinary CO_2 tension.

Effects of THAM

It would seem appropriate in this symposium that the effects of THAM should be mentioned. The dearth of definitive publica-

tions precludes anything beyond preliminary evaluation; moreover chemical methodology has been sufficiently imprecise that few data on actual plasma and urine concentrations of THAM are available. This compound has been used for many years as a buffer in the stabilization of *p*H for biochemical and enzymatic reactions. It has been known for some time that intravenous administration of the compound is followed by the prompt appearance in the urine of a buffer having the physico-chemical characteristics of THAM, suggesting that it was not very well reabsorbed.[61] Solutions usually administered are extremely alkaline and the compound is essentially in the form of the non-ionized base. In body fluids, it may be expected to titrate H^+, thus $(CH_2OH)_3CNH_2 + H^+ \rightleftharpoons (CH_2OH)_3CNH_3^+$ and at *p*H 7.40, its *p*Ka of about 8.2 would indicate that 14 per cent is unionized while 86 per cent is in the form of cation. Since the major source of hydrogen ion in plasma is the CO_2, H_2CO_3/HCO_3^- system, it may be assumed that acutely reaction 1 is shifted to the right by the addition of the alkaline THAM to form cationic THAM + HCO_3^- from CO_2. Accordingly one would expect the CO_2 tension of body fluids to fall and $[HCO_3^-]$ to rise. In the final analysis, buffering of the alkaline THAM must be shared by all body fluids, as discussed in the description of the extrarenal effects of metabolic alkalosis. H^+ is probably mobilized from cells and bone and intracellular *p*H tends to increase. It is not known whether THAM enters cells or not, but it could reduce cellular $[H^+]$ even without entry.

Recent studies in nomal humans receiving large amounts of this compound intravenously indicate that effects similar to the metabolic alkalosis induced by $NaHCO_3$ are observed. The $[HCO_3^-]$, *p*H, and P_{CO_2} of arterial plasma are increased, and pulmonary CO_2 dissipation is reduced, indicating respiratory compensation. In all probability, intracellular *p*H is either normal or slightly increased despite the elevated arterial P_{CO_2}, as discussed previously in the case of metabolic alkalosis. Exhibition of the compound is associated with a prompt increase in urine flow, urine *p*H, and in the rates of excretion of Na^+, K^+, Cl^- and HCO_3^-.

In the absence of precise data on the mechanism of renal excretion, explanation for this must be speculative. The inference that the clearance of this compound is high would lead to the prediction that a large fraction of the load administered would be rapidly excreted. Amounts as large as 300 μM/minute are conventionally administered and this amount *per se* would impose a large osmotic load on the kidney if substantial amounts are nonreabsorbable. In addition, at urine *p*H's below the limit of about 8.2, at least 50 per cent of the THAM is cationic and must be excreted with an anion.

The mechanism for the increase in bicarbonate excretion probably can be accounted for by the increase in filtered load, resulting from increase in plasma $[HCO_3^-]$. The observation that HCO_3^- reabsorption is also increased does not necessarily imply that H^+ extrusion is increased, but only may indicate that some of the H^+ has been diverted from titration of buffer and NH_3. The observed increases in Na^+, K^+ and Cl^- might be tentatively explained on the basis of a reduction in Na^+, H^+ exchange throughout the nephron, secondary to a reduced cellular $[H^+]$, K^+ extrusion increasing as a result of the change in cellular $[H^+]/[K^+]$ in the segment where the movement of these ions is coupled. Should it turn out that THAM penetrates beyond ECF, the possibility of the process of nonionic diffusion playing a role in its excretion may also have to be considered.

The value of this compound as an adjunct to the treatment of respiratory acidosis awaits definition. In essence, its exhibition represents the imposition of a metabolic alkalosis, of a duration determined by the renal excretory rate for the buffer, upon a preexisting respiratory acidosis. That the initial binding of H^+ ions to buffer, the reduction in P_{CO_2} and the elevation in ECF $[HCO_3^-]$ can and do restore plasma *p*H toward normal is undeniable. Simultaneously, however, CO_2 production proceeds apace at $\pm$ 25 mM/minute and, at least in normals, the rate of excretion of CO_2 thru the lungs is reduced. Whether pulmonary CO_2 output is likewise reduced in respiratory acidosis, or whether the alterations in arterial blood *p*H and/or P_{CO_2} change the sensitivity of the respiratory center and promote more effective alveolar ventilation is not clear. In

ly case, CO_2 balance at a more normal terial plasma P_{CO_2} will require that the increment in renal CO_2 loss induced by THAM greater than any decrement in pulmonary excretion of the gas.

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