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THE RENAL REGULATION OF ACID BASE BALANCE WITH SPECIAL REFERENCE TO THE MECHANISM FOR ACIDIFYING THE URINE[1]

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LARGE quantities of acid are continuously produced in the body by the metabolism of the various foodstuffs, yet in health the hydrogen ion concentration of the body fluids is maintained remarkably constant. This regulation of balance between the acidic and basic constituents of the body fluids is dependent upon both respiratory and renal homeostatic mechanisms. In a quantitative sense the rate of production of carbonic acid, amounting to about 20 mols per day, far exceeds the rate of production of other metabolic acids. But because of the volatility of its anhydride, carbon dioxide, carbonic acid is readily and rapidly eliminated by the lungs. Less than one one-hundredth of this quantity of phosphoric and sulfuric acid is produced each day, yet the excretion of these acids, which is effected largely by the kidneys, is in some ways a greater problem than is the excretion of the much larger quantities of carbonic acid. Rarely does any disease process lead to a disturbance of acid base balance because it interferes with the elimination of carbon dioxide in the lungs.

[1] Presented as an Abraham Flexner Lecture at Vanderbilt University School of Medicine on April 20, 1945, and as a Lecture in Medicine at the University of Utah School of Medicine on May 18, 1945.

In contrast at least two disease processes lead to disturbances of acid base balance because they interfere with the adequate elimination of acid by the kidneys. Thus in severe uncontrolled diabetes the excessive metabolic production of beta-hydroxybutyric and acetoacetic acid so overwhelms the normal renal mechanisms for excretion of acid that acidosis results. On the other hand in chronic diffuse glomerulonephritis the kidneys are so damaged that their reduced capacity to excrete acid is inadequate to cope with the normal metabolic production of sulfuric and phosphoric acid, and acidosis results.

In this lecture I propose first, to review briefly the contribution of the renal acid excreting mechanisms to the regulation of acid base balance; second, to present some recent experiments[2] designed to elucidate the nature of the mechanism for acidifying the urine; and third, to point out how the inadequate functioning of these mechanisms in diabetes and in chronic renal disease leads to the development of clinical acidosis.

Let us first review briefly the quantitative aspects of the renal excretion of acid in the normal healthy adult. In the metabolism of 100 gm of protein and 100 gm of fat the normal individual produces phosphoric and sulfuric acid in amounts equivalent to 1,500 cc of N/10 acid. The average diet contains enough available base to neutralize about half of this acid. The remaining half must be neutralized by base derived from bicarbonate of the body fluids. If this excess acid were excreted as neutral sodium salts, the body stores of available base would be rapidly depleted. Accordingly two renal mechanisms have been developed for the excretion of acid without the loss of equivalent amounts of fixed base. First, the renal tubules synthesize ammonia, and convert the sodium salts of the glomerular filtrate into ammonium salts. An equivalent amount of sodium is retained in the body. Second, the renal tubules convert the slightly alkaline glomerular filtrate into an acid urine, and because the acids are excreted partly in the free form, base is retained in the body.

The most acid urine which the kidney can elaborate has a pH of 4.8. At this pH only insignificant amounts of strong acids such as sulfuric and hydrochloric can exist in free form. Hence conservation of base in the excretion of these strong acid anions must be brought about by the conversion of their sodium salts to ammonium salts. But weak acids such as phosphoric, uric and beta-hydroxybutyric can exist in considerable proportion in free form in urine of pH 4.8. Hence conservation of base in the excretion of these weak acid anions can be brought about by the acidification of the urine.

[2] The data summarized in this paper are presented in detail in the *Am. Jour. Physiol.*, 144: 239, 1945.

TABLE 1

MECHANISMS BY WHICH ACID IS EXCRETED WITHOUT LOSS OF EQUIVALENT AMOUNTS OF FIXED BASE. SUMMARY OF QUANTITATIVE DATA ON MAN

	cc N/10 acid/day	Ratio $\frac{NH_3}{acid}$
Normal man:		
1. Excretion of acid combined with NH_3	300–500	1–2.5
2. Excretion of titratable acid	100–300	
Diabetic acidosis:		
1. Excretion of acid combined with NH_3	3,000–5,000	1–2.5
2. Excretion of titratable acid	700–1,500	
Nephritic acidosis:		
1. Excretion of acid combined with NH_3	5–150	0.2–1.5
2. Excretion of titratable acid	20–200	

As is shown in Table 1, compounded from studies of Peters and Van Slyke,[3] Gamble[4] and others, a normal individual excretes the equivalent of 300 to 500 cc of N/10 acid each day in combination with ammonia and 100 to 300 cc as free titratable acid. This titratable acid is largely phosphoric in the form of monobasic phosphate.

The uncontrolled diabetic produces much more metabolic acid than does the normal individual. Not only does he produce phosphoric and sulfuric acids but in addition he produces the ketone bodies beta-hydroxybutyric and acetoacetic acid in extremely large amounts. Total acid production may amount to the equivalent of 5,000 to 10,000 cc of N/10 acid per day. Accordingly the excretion of acid combined with ammonia and the excretion of free titratable acid are greatly increased, as is shown in this table. But despite this tremendous increase in the excretion of ammonia and titratable acid, the renal elimination of acid fails to keep pace with the metabolic production of acid. Ketone bodies are excreted in part as neutral sodium salts and the alkali reserve of the body is depleted.

In nephritis the production of metabolic acid is within normal limits, but the capacity of the kidneys to eliminate acid without loss of base is reduced. As is shown in this table, the ability of the diseased kidney to form ammonia and to acidify the urine is less than that of the normal kidney. The more long standing and severe the disease process the greater is the reduction in functional renal capacity. If the nephritic patient continues to produce some 400 to

[3] J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry." Vol. 1, "Interpretations." Baltimore: Williams and Wilkins Co. 1932.

[4] J. L. Gamble, "Chemical Anatomy, Physiology and Pathology of Extracellular Fluid." A lecture syllabus. Department of Pediatrics, Harvard Medical School, Boston, 1941.

800 cc of N/10 acid per day in excess of the daily intake of available base, yet is incapable of excreting this excess as free acid and as acid combined with ammonia, it is obvious that his body stores of available base will be rapidly exhausted. In fact in the terminal stages of nephritis the total excretion of acid may drop to 25 cc per day, 5 cc combined with ammonia and 20 cc as free titratable acid. Under conditions such as these the alkali reserve of the body could be exhausted in less than one week.

In the absence of renal disease, both in the normal individual and in the uncontrolled diabetic, the quantity of acid excreted in combination with ammonia exceeds that excreted as free titratable acid. Thus the ratio of ammonia to titratable acid varies between limits of 1 and 2.5 (*c.f.* Table 1). In chronic renal disease the capacity of the kidney to excrete ammonia is reduced to a greater extent than is the capacity to excrete an acid urine. Thus the ratio of ammonia to titratable acid decreases. Terminally in some instances the ratio may return toward normal, not as a result of any increase in ammonia excretion, but as a result of extreme depression of the elimination of free acid.

is secreted into the urine, but some leaks out into the renal venous blood.

The nature of the precursor of ammonia has, in the past, been a subject for considerable controversy. Recently, however, ingenious experiments of Dr. Van Slyke and his associates[6] have demonstrated conclusively that 60 per cent. or more of the urinary ammonia is derived from plasma glutamine, and 40 per cent. or less is derived from plasma amino acid. The kidney contains an enzyme, glutaminase, which rapidly splits off the amide nitrogen of glutamine to form glutamic acid and ammonia. This reaction is illustrated in Table 2. Another enzyme, amino acid oxidase, brings about the oxidative deamination of amino acids to form corresponding keto acids and ammonia. In this table alanine is used as an example, although presumably any amino acid could serve as the precursor of ammonia.

The nature of the renal mechanism for acidifying the urine is less well understood than is that for excretion of ammonia, and has never been subjected to any critical experimental study. It is generally conceded that some process is involved such as that illustrated in the equation at the bottom of Table 2. Thus

TABLE 2

RENAL REACTIONS INVOLVED IN THE SECRETION OF AMMONIA AND IN THE ACIDIFICATION OF THE URINE

Ammonia secretion

1. Glutamine $HOOC \cdot CH(NH_2) \cdot CH_2 \cdot CH_2 \cdot CO(NH_2) + H_2O$ → (*Renal Glutaminase*) Glutamic Acid $HOOC \cdot CH(NH_2) \cdot CH_2 \cdot CH_2 \cdot COOH$ + Ammonia NH_3

2. Alanine $HOOC \cdot CH(NH_2) \cdot CH_3 + O$ → (*Renal Amino Acid Oxidase*) Pyruvic Acid $HOOC \cdot CO \cdot CH_3$ + Ammonia NH_3

Acidification of urine

3. *Dibasic Phosphate* Na_2HPO_4 + *Carbonic Acid* H_2CO_3 → *Monobasic Phosphate* NaH_2PO_4 (Excreted) + *Bicarbonate* $NaHCO_3$ (Reabsorbed)

Let us consider the known facts concerning the renal mechanisms for ammonia excretion and for acidifying the urine. In 1921 Nash and Benedict[5] demonstrated quite simply that the renal tubular cells synthesize ammonia and secrete it into the tubular lumen. Thus they observed that the concentration of ammonia in the arterial blood is vanishingly low; that the blood ammonia is unchanged in acidosis when the excretion of ammonia is much increased; and that the quantity of ammonia excreted far exceeds the quantity filtered through the glomeruli. Furthermore, the concentration of ammonia in the blood leaving the kidney is always higher than that in the blood entering the kidney. Therefore it is apparent that ammonia must be formed in the kidney from some precursor in the arterial blood. Most of this ammonia carbonic acid reacting with dibasic phosphate forms monobasic phosphate and sodium bicarbonate. Base is conserved by the reabsorption of sodium bicarbonate and free titratable acid is excreted in the urine in the form of monobasic phosphate. Although this reaction adequately describes the end result of urinary acidification, it does not indicate the means by which this end is achieved. Indeed three distinct theoretical mechanisms have been postulated, each based fundamentally upon this reaction.

In Fig. 1 the three current theories as to the nature of the mechanism for acidifying the urine are illustrated in highly diagrammatic fashion. The kidney is represented as a single renal unit composed of glomerulus and tubule. Such a simplified representation

[5] T. P. Nash and S. R. Benedict, *Jour. Biol. Chem.* 48: 463, 1921.

[6] D. D. Van Slyke, R. A. Phillips, P. B. Hamilton, R. M. Archibald, P. H. Futcher and A. Hiller, *Jour. Biol. Chem.*, 150: 481, 1943.

is permissible for the million or so nephrons comprising each kidney are functionally identical in a qualitative sense. In each glomerulus there is formed an ultrafiltrable of plasma; the pH of the filtrate is the same as that of the plasma, namely, pH 7.4. In its passage down the tubule this filtrate is elaborated into acid urine of pH 4.8 or higher.

According to the *phosphate reabsorption theory* the significant constituents of the glomerular filtrate are monobasic and dibasic phosphate, present in the original filtrate in a ratio of 1 part of the monobasic salt to 4 parts of the diabasic salt. It is presumed that

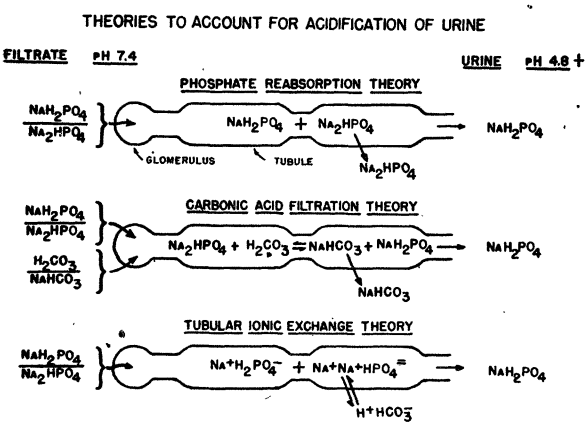

FIG. 1. From Pitts, R. F. and Alexander, R. S., *Amer. Jour. Physiol.*, 144: 239, 1945.

the diabasic phosphate is reabsorbed by the renal tubules and returned to the postglomerular blood, whereas the monobasic phosphate is excreted and constitutes the titratable acid of the urine. If the urine contained only monobasic phosphate it would have a pH of approximately 4.8, and for each millimol of phosphate excreted 8/10th of a milliequivalent of titratable acid would be eliminated. This theory is implied in a number of text-books of biochemistry.

According to the *carbonic acid filtration theory* the glomerular ultrafiltrate not only contains phosphate but also carbonic acid and sodium bicarbonate. Sendroy, Seelig and Van Slyke[7] developed this theory on the assumption that the renal tubules are completely impermeable to carbonic acid and that they actively reabsorb sodium bicarbonate. Accordingly the reaction between dibasic phosphate and carbonic acid occurring within the tubular lumen is forced to the right by the reabsorption of bicarbonate. The monobasic phosphate which is formed is excreted in the urine as titratable acid.

According to the *tubular ionic exchange theory,* a tubular secretory mechanism is responsible for the elimination of acid in the urine, not a filtration-reabsorption mechanism as is implied in the first two theories. The significant constituents of the glomerular filtrate are considered to be monobasic and dibasic phosphate. Bicarbonate and carbonic acid are presumed to be reabsorbed in large part by the renal tubules, and therefore need not be considered. In the passage of the filtrate down the tubules, hydrogen ions derived from the tubular cells are exchanged for sodium ions in the tubular lumen. Dibasic phosphate is thereby converted to monobasic phosphate, and base is returned to the blood. The source of the hydrogen ions is presumably carbonic acid formed within the renal tubular cells. This theory was originally advanced by Homer Smith.[8]

It is significant that any one of these three theories is adequate to explain the titratable acidity and the pH of normal urine. Yet no critical experiment has appeared in the literature which either proves one theory or rules out another. Since the mechanism for acidifying the urine is a major renal homeostatic mechanism and since failure of the adequate excretion of acid in disease leads to the development of clinical acidosis, it seemed worth while to attempt to determine the nature of this mechanism.

It occurred to us that it should be possible to test these three theories experimentally, for each has inherent within it a specific identifiable limitation of its capacity to cause the excretion of acid. You will note in the *phosphate reabsorption theory* and in the *carbonic acid filtration theory* that all the acid excreted in the urine was originally present in the glomerular filtrate. In the first theory monobasic phosphate constitutes the filtered acid, and the quantity of this compound which appears in the urine as titratable acid is limited by the capacity of the renal tubules to reabsorb the dibasic phosphate which accompanies it. In the second theory carbonic acid constitutes the filtered acid, and the quantity of titratable acid excreted can not exceed the quantity of carbonic acid originally filtered. These two theoretical mechanisms are therefore limited by the measurable capacity of the tubules to reabsorb and by the measurable capacity of the glomeruli to filter. In sharp contrast the *ionic exchange mechanism* is limited by the measurable capacity of the tubules to secrete. The acid appearing in the urine is not that which was originally filtered but is that which the renal tubules have added to the filtrate by a type of secretory process, namely, ionic exchange.

We have tested these three theories in unanesthetized dogs in the following manner. The renal mecha-

[7] J. Sendroy, Jr., S. Seelig and D. D. Van Slyke, *Jour. Biol. Chem.*, 106: 479, 1934.

[8] H. W. Smith, "The Physiology of the Kidney." New York: Oxford University Press. 1937.

nism for acidifying the urine was stimulated by the repeated feeding of dilute hydrochloric acid. After the establishment of a suitable acidosis the animals were provided with a large excess of phosphate in continuous intravenous infusions. The rate of excretion of titratable acid in the urine was then compared with the rate of reabsorption of phosphate and with the rate of filtration of carbonic acid. It was found that the observed rate of excretion of acid far exceeds that which can be explained by the *phosphate* or *carbonic acid theories*. Since monobasic phosphate and carbonic acid are the only acids present in the glomerular filtrate in significant amounts, it is evident that acid must be added to the filtrate by some type of tubular secretory mechanism. We are convinced that the *ionic exchange theory* adequately describes that mechanism.

I should like to present these experiments for your critical appraisal, but let us first review briefly the methods by which we propose to solve our problem. It is obvious that the key to the solution lies in knowing the rate at which plasma is filtered through the glomeruli, for if we know the glomerular filtration rate and simultaneously determine the concentration of carbonic acid and phosphate in the plasma, we can readily calculate the rate at which these materials are filtered through the glomeruli. Dr. Smith and his associate have given us in the creatinine clearance a ready accurate means of determining the rate of filtration of plasma through the glomeruli of the dog. In Fig. 2 we have illustrated the principles involved in a quantitation of the rate of reabsorption of phosphate. The same principles may of course be applied to carbonic acid. The rate of filtration of phosphate is obviously the product of the rate of filtration of plasma through the glomeruli, as measured by the creatinine clearance, and the concentration of phosphate in each cc of that plasma. The rate of excretion of phosphate in the urine is the product of the urine flow in cc per minute and the urine phosphate concentration. The rate of phosphate reabsorption is obviously equal to the rate of filtration minus the rate of excretion.

Our experiments have been performed on trained unanesthetized female dogs, chosen for their even dispositions and ready cooperation. Urine was collected by catheter and blood samples were drawn from the femoral artery. Table 3 summarizes the significant data obtained in one experiment consisting of four identical clearance periods. It is apparent from the first column that this dog had a glomerular filtration rate slightly more than half that of an adult human. The next two columns indicate that the animal had a moderate acidosis, produced by the repeated feeding of hydrochloric acid. The normal carbon dioxide content of dog plasma, like that of the human, ranges between 23 and 26 millimols per liter, so that values of 18 to 19 millimols per liter are indicative of moderate reduction and hence of moderate acidosis. Arterial pH was determined directly by a hypodermic type glass electrode, and as you see, the observed values are slightly below the normal of pH 7.4, again indicative of moderate acidosis. The plasma phosphate concentration was elevated to some 8 to 9 times the normal by the continuous intravenous infusion of neutral sodium phosphate. The next two columns, the rate of phosphate reabsorption and rate of carbonic acid filtration, were determined by the methods we have just described. The urine pH, and in the last column, the titratable acid of the urine were

TABLE 3

AN EXPERIMENT ON AN ACIDOTIC DOG DESIGNED TO TEST CRITICALLY THE SEVERAL THEORIES OF THE NATURE OF THE RENAL MECHANISM FOR ACIDIFYING THE URINE

Rate of glomerular filtration cc/min.	Arterial plasma concentration			Rate of phosphate reabsorption mM./min.	Rate of H_2CO_3 filtration mM./min.	Urine pH	Rate of excretion		
	Total CO_2 mM./L.	pH	Phosphate mM./L.				Phosphate mM./min.	Creatinine mM./min.	Titratable acid mEq./min.
73.1	18.7	7.34	8.37	0.123	0.075	6.06	0.489	0.171	0.310
76.4	18.7	7.34	8.41	0.128	0.078	6.02	0.514	0.168	0.340
76.9	18.6	7.37	8.53	0.131	0.073	6.26	0.525	0.163	0.298
75.1	18.5	7.39	9.00	0.138	0.068	6.32	0.538	0.166	0.297

THE MEASUREMENT OF THE RATE OF REABSORPTION OF PHOSPHATE

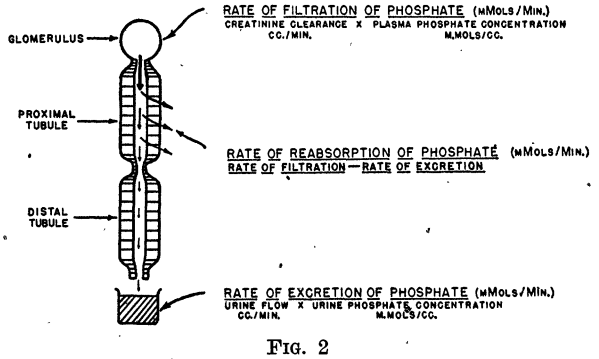

FIG. 2

determined electrometrically, that is, the urines were titrated from their observed pH to the pH of the plasma, using the glass electrode as the measuring instrument. This titration therefore represents the reversal of the process of acidification of the urine which was performed by the kidney. The average

TABLE 4

FORMS OF THE HENDERSON-HASSELBALCH EQUATION USED IN THE ANALYSIS OF THE DATA

1. $pH = pK' + \text{Log } \frac{(salt)}{(acid)}$
2. $pH = 6.8 + \text{Log } \frac{(Na_2HPO_4)}{(NaH_2PO_4)}$
3. $pH = 6.1 + \text{Log } \frac{(NaHCO_3)}{(H_2CO_3)}$
4. $pH = 4.97 + \text{Log } \frac{(Creatinine)}{(Creatinine\text{–}HCl)}$

excretion of titratable acid in this experiment is 0.311 milliequivalents per minute. In more familiar terms this is equivalent to the excretion of 4,500 cc of N/10 titratable acid per day, which is some 3 times the maximum ever observed in severe diabetic acidosis in man. The factor which accounts for this high rate of excretion of titratable acid is the very high rate of excretion of phosphate.

In order to analyze the data further we have applied the Henderson-Hasselbalch equation in the several ways which we shall describe presently. The general form of this equation is given in the top line of Table 4. Since in our experiments we must deal with three buffers—phosphate, bicarbonate, and creatinine—we must apply the equation in the three forms listed below. We have directly measured the pH and the total concentration of these three buffers in both blood and urine. From these measurements it is possible to calculate the concentration of the acidic and basic component of each buffer and the absolute contribution of the acid component of each buffer to the titratable acidity of the urine.

TABLE 5

A CRITICAL ANALYSIS OF THE DATA FROM TABLE 3 WHICH INDICATES THE INADEQUACY OF THE PHOSPHATE AND CARBONIC ACID THEORIES AND THE NECESSITY FOR POSTULATING SOME TYPE OF TUBULAR SECRETORY MECHANISM FOR ACIDIFYING THE URINE

Titratable acid of urine						
Observed	Calculated from total buffer excreted		Calculated from phosphate reabsorption theory		Calculated from carbonic acid filtration theory	
mEq./min.	mEq./min.	Per cent. of observed	mEq./min.	Per cent. of observed	mEq./min.	Per cent. of observed
0.310	0.312	100.6	0.028	9.0	0.075	24.2
0.340	0.339	99.7	0.029	8.5	0.078	22.9
0.298	0.300	100.6	0.028	9.4	0.073	24.5
0.297	0.300	101.0	0.028	9.4	0.068	22.9
0.311	0.313	100.6	0.028	9.1	0.074	23.6

In Table 5 the data of the preceding experiment have been subjected to rigorous analysis. The first column repeats the observed titratable acidity of the urine. Then applying the Henderson-Hasselbalch equation we calculated the titratable acidity in the three ways noted in the succeeding three pairs of columns. First we calculated the acidity from the measured pH of the urine and from the measured excretion rate of the several buffers. The agreement with the observed acidity is remarkable, averaging a shade over 100 per cent. This calculation is in reality a check on the accuracy of all our measurements, and it merely indicates that our data are good and deserving of further analysis. The quantity of titratable acid which could be excreted if the *phosphate reabsorption theory* were correct is rather low, averaging only 9.1 per cent. of the observed titratable acid. Similarly, the *carbonic acid filtration theory* falls short of explaining the acidity of the urine in this experiment, averaging only 23.6 per cent. of the observed titratable acid.

Four experiments in all were performed on four dogs. Each experiment was identical to that which we have just presented. Table 6 summarizes the

TABLE 6

SUMMARY OF 4 EXPERIMENTS ON ACIDOTIC DOGS ANALYZED ACCORDING TO THE METHOD OUTLINED IN TABLE 5

	Titratable acid of urine						
	Observed	Calculated from total buffer excreted		Calculated from phosphate reabsorption theory		Calculated from carbonic acid filtration theory	
	mEq./min.	mEq./min.	Per cent. of observed	mEq./min.	Per cent. of observed	mEq./min.	Per cent. of observed
Dog 5	0.311	0.313	100.6	0.028	9.1	0.074	23.6
Dog 6	0.213	0.218	102.3	0.024	11.1	0.052	24.5
Dog 2	0.307	0.317	103.0	0.032	10.5	0.053	17.3
Dog 1	0.369	0.376	102.0	0.027	7.4	0.064	17.2

average calculated values for each of these four experiments. It is obvious that the *phosphate reabsorption theory* and the *carbonic acid filtration theory* fail completely to explain the titratable acidity observed in our experiments. The *phosphate theory* accounts for only 7 to 11 per cent. of the observed acid; the *carbonic acid theory* accounts for only 17 to 24 per cent. of the observed acid.

(To be concluded)