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FACTORS AFFECTING THE EXCRETION OF TITRATABLE ACID
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THE RENAL REGULATION OF ACID-BASE BALANCE IN MAN. II. FACTORS AFFECTING THE EXCRETION OF TITRATABLE ACID BY THE NORMAL HUMAN SUBJECT ¹

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Transformation of the slightly alkaline glomerular filtrate into acid urine has been assigned in man (1) as in the dog (2) to the exchange of hydrogen ions formed within the tubular cells for ions of fixed base in the tubular urine. The most acid urine which the kidney can form is of pH 4.5 to 4.7 (1, 3). At this reaction negligible quantities of strong acids such as hydrochloric or sulfuric can exist in free form. Accordingly the exchange of hydrogen ions for base bound by chloride and sulfate in the tubular urine is quantitatively insignificant. But weak buffer acids such as monobasic phosphate, uric acid, creatinine, and beta-hydroxybutyric and aceto-acetic acids can exist in considerable proportion in free titratable form in urine of such acidity (4). As a consequence, the exchange of hydrogen ions for base bound by weak buffer acids in the tubular urine plays a significant role in stabilizing the alkali reserve of the body (5).

It has been demonstrated in the dog that the major determinants of the rate of excretion of titratable acid are (1) the rate of excretion of buffer; (2) the acid strength of the buffer excreted; and (3) the degree of acidosis, as reflected in the bicarbonate content of the plasma (6). Each of these factors appears to play a role in clinical acidosis in man. Thus, in severe diabetic ketosis, a high rate of excretion of titratable acid is associated with a low alkali reserve and with a high rate of excretion of ketone bodies (4). The ketone bodies are relatively strong buffer acids. Accordingly the kidney can salvage from them only a portion of the base they bind in the plasma and glomerular filtrate, and although the rate of titratable acid excretion is high in diabetic

ketosis, a considerable complement of base is lost in the urine.

In the present study the role played by these several factors mentioned above has been investigated systematically in normal human subjects in whom acidosis has been induced by the ingestion of ammonium chloride. By infusing phosphate, creatinine and para-aminohippurate at progressively increasing rates to cause the elimination in the urine of increasing quantities of these buffers, it has been found in man as in the dog that (1) the rate of excretion of titratable acid increases in proportion to the rate of excretion of buffer; (2) at any given molar rate of excretion of buffer the quantity of titratable acid eliminated per unit of time is greatest for the buffer of lowest acid strength (phosphate) and least for the buffer of greatest acid strength (para-aminohippurate); and (3) the rate of excretion of titratable acid is, within limits, proportional to the degree of reduction of bicarbonate in the plasma.

METHODS

The 12 experiments forming the basis of this report were performed on 3 normal adult male subjects. However, the data presented in this paper have been restricted to those obtained on a single subject on whom a complete set of experiments was performed. Additional experiments on the other 2 subjects confirmed the observations recorded here. Experimental methods employed were reported in the preceding paper (1).

RESULTS

The relationship between the rate of excretion of titratable acid and the rate of excretion of buffer

It is generally recognized that the majority of the titratable acid of normal human urine is monobasic phosphate, and that changes in the rate of excretion of endogenous phosphate are accom-

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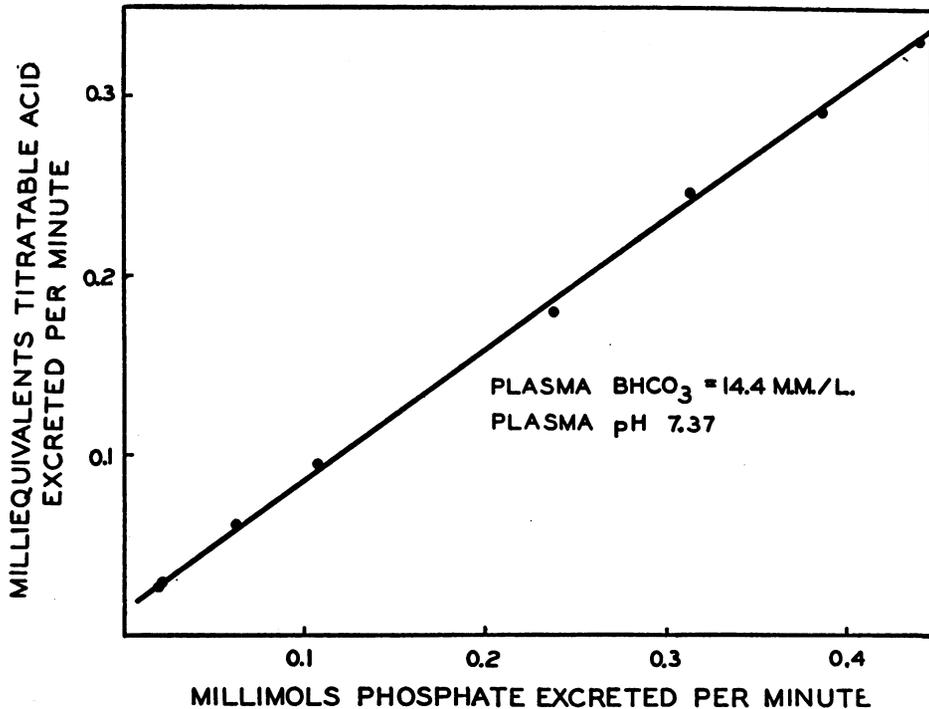


FIG. 1. THE RELATION BETWEEN THE RATE OF EXCRETION OF PHOSPHATE AND THE RATE OF EXCRETION OF TITRATABLE ACID IN MAN IN ACIDOSIS

Data from an experiment on subject R. F. P.

panied by equivalent changes in the rate of excretion of titratable acid (5). In Figure 1 are presented the results of a systematic study of the relationship between urinary phosphate and titratable acid, carried out over a far wider range of phosphate excretion than ever occurs spontaneously. The data are derived from Experiment 1 in Table I of the preceding paper (1). The subject was in a state of moderate acidosis characterized by a plasma bicarbonate concentration of 14.4 mM per liter and a plasma pH of 7.37. The first 2 points in the lower left-hand corner of this figure represent control rates of excretion of titratable acid prior to the administration of phosphate. About 50 per cent of the acid eliminated in these 2 periods can be accounted for as monobasic phosphate derived from endogenous stores. The remainder is creatinine and other organic acids. Neutral sodium phosphate (pH 7.4)² was then infused at progressively increasing rates to increase

² We are indebted to the William R. Warner Co. of New York for the preparation of a generous supply of ampoules of the sterile pyrogen-free sodium phosphate solution of pH 7.4 used in this work.

the rate of phosphate excretion to a maximum of 0.443 mM per minute. The rate of excretion of titratable acid increased in direct proportion to the rate of excretion of phosphate. Throughout the entire series of observations the urine reaction varied only between pH 4.49 and 4.65. Thus phosphate, which entered the glomerular filtrate mainly in the dibasic form, was eliminated in the urine entirely in the monobasic form. Indeed at such acidities traces of free phosphoric acid are excreted. These results in man differ slightly from those observed in the dog, in which a curvilinear relationship was noted between the rates of excretion of titratable acid and phosphate (6). Deviation from a linear relationship indicates that the kidney of the dog is unable to elaborate as acid a urine at high rates of phosphate excretion as at low rates. This fact is in accord with the statement in the preceding paper (1), that the renal tubular capacity of the dog to acidify the urine is less than that of man. The proportionality between the rate of excretion of acid and the rate of excretion of buffer which is evident in Figure 1 for phosphate, is equally true for other buffers (*cf.* Figure 2).

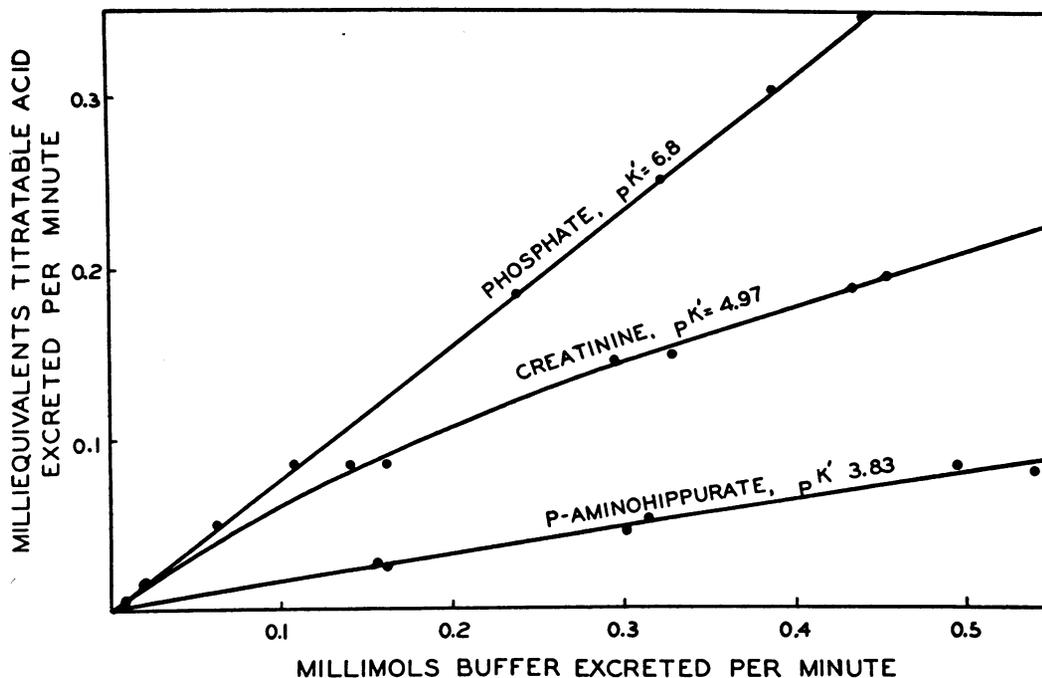


FIG. 2. A COMPARISON OF THE EFFECTIVENESS OF SEVERAL URINARY BUFFERS IN MAN IN ACIDOSIS

All data on subject R. F. P.

The relationship between the rate of excretion of titratable acid and the acid strength of the urinary buffer

Beta-hydroxybutyric acid is a relatively strong buffer acid, and in solutions of pH 4.7 half exists as free acid and half as neutral salt. In contrast, the second hydrogen of phosphate is only weakly acidic, and in solutions of this reaction, phosphate exists entirely in the titratable monobasic form. Since the reaction of the urine is limited to an acidity no greater than pH 4.5 to 4.7 it obviously follows that the stronger the buffer acid, the less completely can the renal tubules exchange hydrogen ions for ions of fixed base bound by that buffer in the tubular urine. Accordingly, when large quantities of beta-hydroxybutyrate are excreted in the urine in diabetic ketosis, sufficient base is lost to deplete the body reserves and lead to acidosis.

In Figure 2 is presented a comparison of the rates of excretion of titratable acid at a series of equivalent molar excretion rates of phosphate, creatinine and para-aminohippurate. The extent of the acidosis was essentially the same in the

3 experiments, for plasma bicarbonate concentrations varied only from 13 to 15 mM per liter. In each instance the rate of excretion of titratable acid was corrected by subtracting from the observed value that acid due to buffers other than the one specifically studied. For example, in the experiments on phosphate, the titratable acid due to the excretion of endogenous creatinine and organic acid was deducted from the total; in the experiment on para-aminohippurate, the titratable acid due to phosphate was also deducted. Such corrections are appreciable only at low rates of buffer excretion; they obviously account for the fact that all curves start at the origin. These corrections have been applied to the data presented in Figures 2, 3 and 4, but not to the data in Figure 1.

It is evident from Figure 2 that the rate of excretion of titratable acid at any given rate of excretion of buffer is determined by the acid strength of the buffer. Thus, secondary phosphate, the weakest acid buffer ($pK' = 6.8$), yields the greatest proportion of base in exchange for hydrogen ions; and para-aminohippurate, the strongest acid buffer ($pK' = 3.83$), yields the least proportion

of base in exchange for hydrogen ions. Creatinine, a buffer of intermediate strength ($pK' = 4.97$), occupies an intermediate position in the series. Beta-hydroxybutyrate ($pK' = 4.70$) would fit into this series between creatinine and para-aminohippurate somewhat closer to the former than to the latter. Primary phosphate is such a strongly acid buffer ($pK' = 2.11$) that the kidney can exchange hydrogen ions for the base which it binds only in insignificant trace amounts. Were they plotted in this diagram, such points would fall on or very near the base line.

The relation between the rate of excretion of titratable acid and the extent of the acidosis

It has been repeatedly observed that the ingestion of sufficient acid to depress the plasma bicarbonate level results in the prompt excretion of urine of low pH, and in the elimination of increased quantities of titratable acid (7). In Figure 3 are presented results of 2 experiments with creatinine, identical throughout except for the fact that in one a moderately severe acidosis had been induced, while in the other acid base balance was within limits of normal. It is evident at any given rate of excretion of buffer that the rate of excretion of titratable acid is greater in acidosis than when acid base balance is normal. What is surprising is the rather high rate of excretion of acid which is attained when the alkali reserve is within normal limits. Even more surprising are the rather small

differences in the rates of excretion of titratable acid in similar paired experiments in which phosphate was infused, cf. Figure 4. In the experiment in which no ammonium chloride was ingested, the plasma bicarbonate level was within the accepted range of normal (24.2 mM per liter), yet the infusion of phosphate was accompanied by increases in titratable acid excretion nearly the same as those observed in moderately severe acidosis. It seems reasonable to interpret these results as meaning that plasma bicarbonate concentrations within a range accepted as normal, characterize a state of mild acidosis so far as the kidneys are concerned. Furthermore, stimulation of the tubular exchange of hydrogen ions for sodium ions must be nearly maximum under such conditions of mild acidosis. Indeed, experiments to be presented in a subsequent paper on bicarbonate indicate that maximum stimulation of acid excretion is effected by reducing plasma bicarbonate to approximately 20 mM per liter.

A fact which is not evident from the data presented in these charts is that the excretion of increased quantities of buffer actually stimulates the formation of urine of more acid reaction. In the initial control periods of the experiment with phosphate in which the bicarbonate content of the plasma was 24.2 mM per liter, the pH of the urine was just below 7.0. Had this pH been maintained in subsequent periods in which the rate of excretion of phosphate was increased, the rate of excretion of titratable acid would have risen con-

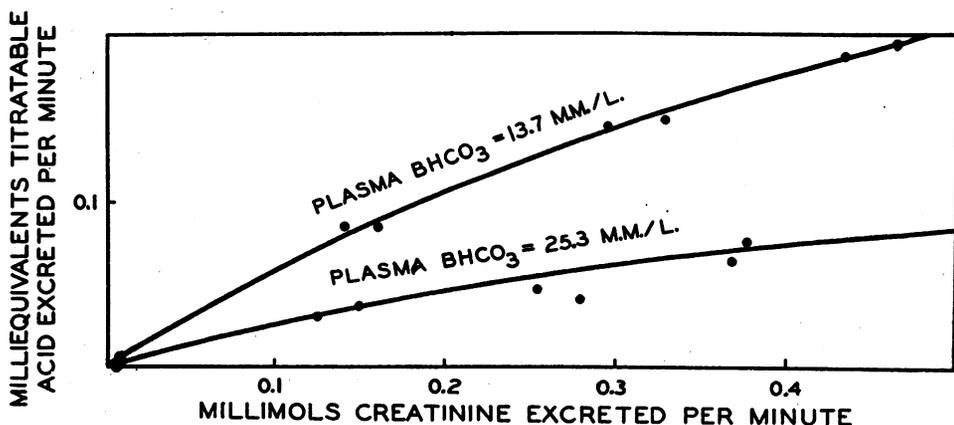


FIG. 3. THE RELATION BETWEEN THE RATE OF EXCRETION OF TITRATABLE ACID AND THE DEGREE OF ACIDOSIS AT A SERIES OF COMPARABLE EXCRETION RATES OF CREATININE
All data on subject R. F. P.

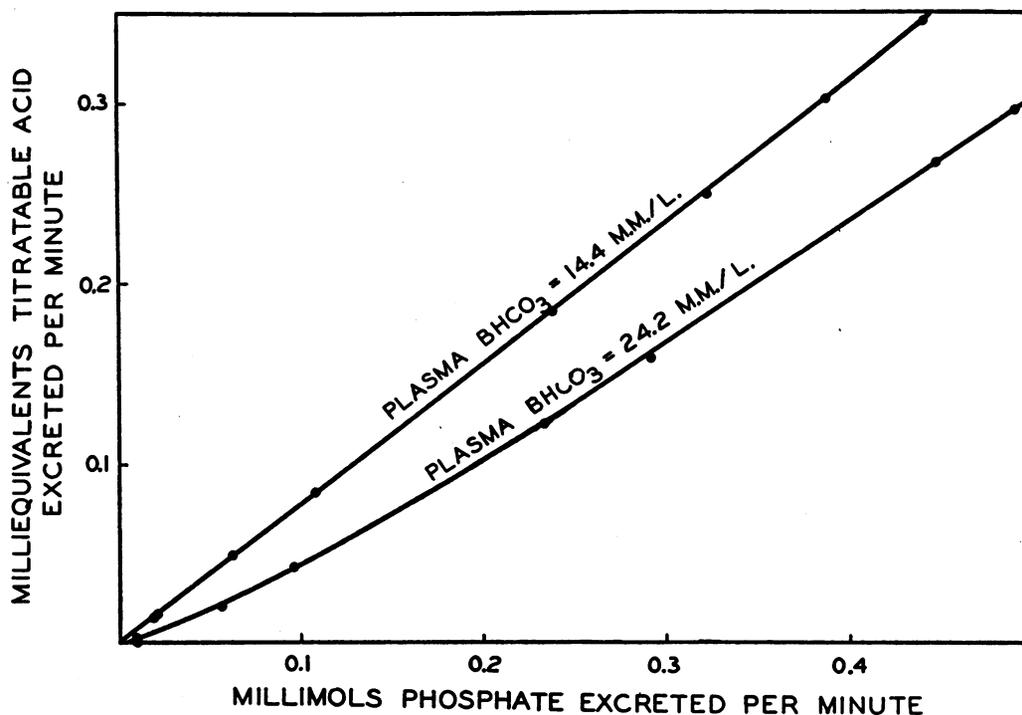


FIG. 4. THE RELATION BETWEEN THE RATE OF EXCRETION OF TITRATABLE ACID AND THE DEGREE OF ACIDOSIS AT A SERIES OF COMPARABLE EXCRETION RATES OF PHOSPHATE

All data on subject R. F. P.

siderably. But the urine actually became more acid during phosphate infusion until at the highest rate of phosphate excretion urine pH dropped below 6.0. The reason for this change in reaction of the urine on the infusion of phosphate is not known, but it has been observed previously in animal experiments (6, 8).

The effect of acidosis on the renal tubular reabsorption of phosphate

Phosphaturia is commonly observed in ammonium chloride acidosis and in the acidosis of diabetic ketosis (9, 10). The excess urinary phosphate is derived in large part from stores of non-diffusible organic acid soluble phosphorus within the cells, hydrolysis of which liberates inorganic phosphate into the plasma (11). According to 1 view, the renal reabsorptive mechanism for phosphate is unaltered in acidosis and the elevation of plasma phosphate which results from hydrolysis of the esters adequately explains the increased rate of phosphate excretion (12). On the other hand, it has been claimed that the plasma

phosphate concentration is below normal in acidosis and that the phosphaturia results from a depression in the capacity of the renal tubules to reabsorb phosphate (13). Since both views are based upon experimental work on dogs neither may be strictly applicable in man. We have accordingly analyzed our data in such a way that we might compare the characteristics of the reabsorptive mechanism in acidosis and in the normal, both at normal and elevated plasma concentrations of phosphate.

In Figure 5, rates of filtration, reabsorption and excretion of phosphate are plotted against the quantity of phosphate filtered per minute. All data were obtained in experiments on a single subject. The values designated as *acidosis* were observed following sufficient ammonium chloride to reduce the plasma bicarbonate concentration to 12 to 15 mM per liter. The values designated as *normal* were observed at plasma bicarbonate levels between 24 and 26 mM per liter. It is evident that as the quantity of phosphate filtered exceeded 0.20 mM per minute, the renal tubular reabsorptive

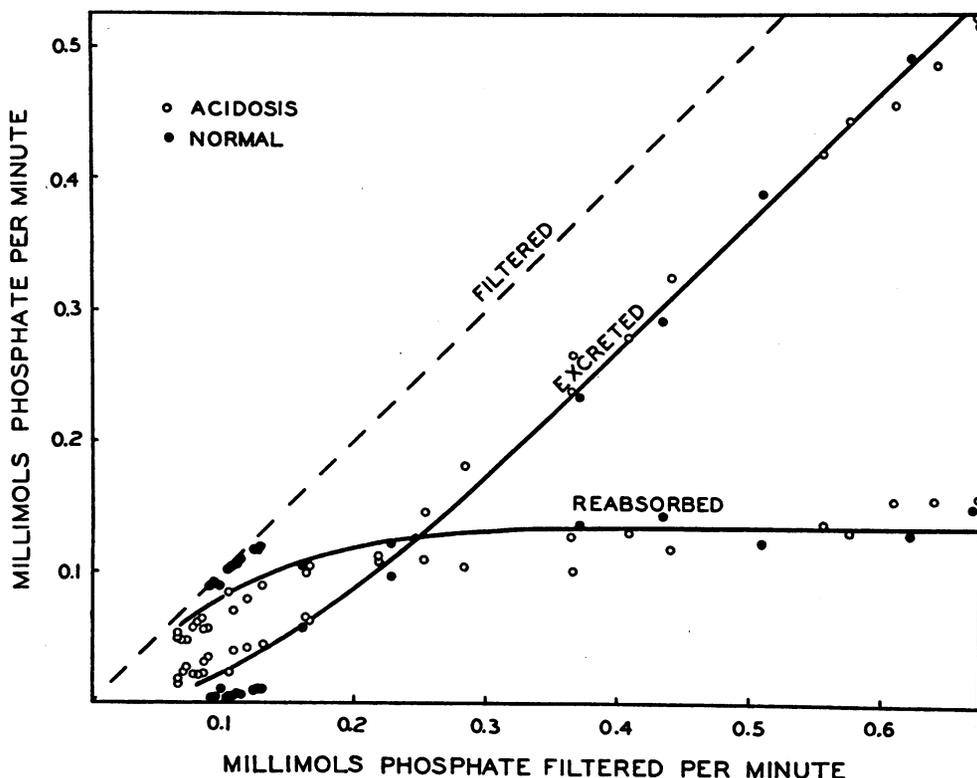


FIG. 5. THE RELATION BETWEEN THE QUANTITY OF PHOSPHATE FILTERED THROUGH THE GLOMERULI AND THE QUANTITIES REABSORBED BY THE RENAL TUBULES AND EXCRETED IN THE URINE

All data on subject R. F. P.

mechanism became saturated and phosphate was returned from tubular urine to blood at an average rate of 0.130 mM per minute. All phosphate filtered in excess of this quantity was excreted in the urine. There appears from our limited data to be no difference between this maximum rate of reabsorption in acidosis and under conditions of normal acid-base balance. In this respect, results on man and dog (12) are in agreement. In contrast, at normal plasma concentrations of phosphate the reabsorption of phosphate is significantly less complete in acidosis than under conditions of normal acid-base balance. Accordingly, the spontaneous rate of excretion of phosphate is higher in acidosis, a factor no doubt of major importance in explaining the phosphaturia in man. In our experience dogs show much less phosphaturia and no appreciable change in reabsorption of phosphate at normal plasma levels in acidosis (12). In these observations on man there appeared to be no significant effect of acidosis on plasma phosphate con-

centration, the range of variation being about the same in acidosis as in the normal. Apparently diminished reabsorption compensated more or less exactly for increased liberation of phosphate from intracellular stores. However it must be emphasized that the acidosis in our experiments was of short duration (24 hours). In more prolonged states of acidosis plasma phosphate may fall to levels below normal.

DISCUSSION

It is evident from the experiments presented above that a number of interrelated factors limit the quantity of titratable acid which the kidney can excrete. First, the kidney is limited in its capacity to excrete urine containing hydrogen ions in high concentration. This capacity undoubtedly varies somewhat in different normal subjects. Although the accepted limit of acidity is pH 4.7 (3), 2 of our 4 subjects could excrete urine as acid as pH 4.49. Expressed as a hydrogen ion

gradient between urine and plasma, the maximum which the renal tubules of these 2 subjects could establish is 800 to 1. It is obvious that in the absence of buffer the exchange of hydrogen ions for ions of fixed base in the tubular urine could proceed only to an insignificant extent before this limiting gradient would be attained. Second, the quantity of buffer which is eliminated by the normal subject is rather small, and as a consequence the quantity of base which can be salvaged from the urine by hydrogen ion exchange is likewise small. In the normal individual sufficient buffer (largely phosphate) is present in the urine to permit recovery of only 10 to 30 mEq of base each day. In contrast, in diabetic ketosis as much as 500 to 1000 mM of beta-hydroxybutyrate and aceto-acetate may be excreted in 24 hours. Because of the increased availability of buffer, up to 150 mEq of base may be returned to the body each day in exchange for hydrogen ions. Third, the acid strength of the buffer is a limiting factor in the exchange of hydrogen ions for base. Obviously the lower the acid strength, the more completely can the kidney salvage base bound by buffer in urine of maximal acidity. All of the secondary base can be recovered from phosphate, only half can be recovered from beta-hydroxybutyrate. Although titratable acid excretion in diabetic ketosis may increase to some 10 times the normal because of increased buffer excretion, the loss of available base is likewise increased because of the high acid strength of the buffer. Theoretically at least there must be some ceiling to the quantity of hydrogen ions which the kidney can exchange, even under hypothetical conditions of unlimited supplies of buffer of optimum acid strength, for the process requires energy, and the energy available to the kidney is not unlimited. However, in our experiments we have by no means approached any such limiting rate of exchange, although for short periods of time during phosphate infusion hydrogen ions have been transferred at a rate which would be equivalent to 0.6 mol per day. This is equal to the excretion of 6000 ml. of one-tenth normal acid, or to the renal manufacture from a buffer precursor of 50 grams of sodium bicarbonate per day.

As Henderson (14) has so clearly pointed out, the properties of secondary phosphate are such as to render it the ideal urinary buffer. However,

from this fact one must not make the extrapolation that the phosphaturia of diabetic acidosis is compensatory in nature. The kidney can salvage only the secondary base of phosphate, the primary bound base is lost in the urine. Thus, for each extra mol of phosphate excreted, the body store of base is depleted to the extent of 1 equivalent. The only point about the process which can be considered as compensatory is the fact that the hydrolysis of organic intracellular phosphate enables base of the cells to be transferred to the plasma. Thus, the cellular phosphate esters bind roughly the same quantity of base per mol of phosphate as is bound by inorganic phosphate in the plasma. The excretion of this phosphate in inorganic form as monobasic phosphate returns to the plasma the secondary bound base as bicarbonate. Thus, intracellular base replenishes extracellular bicarbonate in acidosis, but in the process more than half the base is lost. The kidney actually operates a little more efficiently on beta-hydroxybutyrate than it does on phosphate, for in severe ketosis less than half the base bound by this hydroxy acid in the plasma is lost in the urine.

The reabsorptive mechanism for phosphate in man appears to be qualitatively similar to that in the dog in that the tubules are capable of reabsorbing only a limited quantity of phosphate per unit of time. When as a consequence of an increase in plasma concentration the quantity delivered into the filtrate exceeds the reabsorptive capacity of the tubules, the excess is excreted in the urine. The reabsorptive capacity is fixed, limited, and independent of plasma concentration when that concentration is sufficiently high to saturate the mechanism. This maximum reabsorptive capacity appears to be unaffected in either dog or man by moderately severe acidosis of short duration. However, at low plasma concentrations the kinetics of the reabsorptive process appear to be altered in man, for reabsorption is only partial and excretion is correspondingly increased. The diminished reabsorption at normal plasma concentrations is undoubtedly a factor in explaining the phosphaturia of acidosis in man. Apparently, in our experiments increased excretion was balanced more or less exactly by increased delivery of inorganic phosphate into the plasma, for we have observed no significant differences in plasma level in man as a consequence of acidosis.

CONCLUSIONS

The rate of excretion of titratable acid in man as in the dog is largely determined by 3 factors, namely: (1) the rate of excretion of buffer; (2) the acid strength of the buffer; and (3) the extent of the reduction in the plasma concentration of bicarbonate. For phosphate and para-aminohippurate the relationship between buffer and titratable acid excretion is essentially a linear one within the range of buffer excretion that we have studied. For creatinine the relationship is curvilinear because urine pH increases slightly at high rates of buffer excretion. At any given molar rate of excretion of buffer the rate of elimination of titratable acid is highest for the buffer of lowest acid strength. The accepted normal range of 24 to 26 mM of bicarbonate per liter of plasma constitutes a state of mild acidosis so far as the kidneys are concerned, and titratable acid elimination proceeds at a nearly maximal rate when a buffer of favorable acid strength is presented to the kidney. Further reduction of plasma bicarbonate to 20 mM per liter maximally stimulates the acid excretory mechanism. The results presented are in accord with the view that the urine is acidified by the exchange of hydrogen ions formed within the tubular cells for ions of fixed base in the tubular urine.

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