# THE INFLUENCE OF URINARY IONIC STRENGTH ON PHOSPHATE $pK_2'$ AND THE DETERMINATION OF TITRATABLE ACID *

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It is well recognized that the pK′ value for the salt of a weak acid or base is significantly influenced by the ionic strength of the solution. In the case of phosphate, the theoretical value for $pK_2$ in an infinitely dilute solution is 7.181 at 37° C. (1) and in a solution of ionic strength comparable to that of plasma is approximately 6.8. The small variations in ionic strength ordinarily encountered in plasma would not be expected to produce significant deviations from this latter value. However, in view of the wider range of ionic strengths which occurs in urine, values differing appreciably from 6.8 might be anticipated.

In the present study an attempt has been made to evaluate the relationship between $pK_2'$ phosphate and ionic strength in urine, and to define the usual range of values which might be encountered in the course of acute phosphate loading experiments. A method has been devised for the determination of phosphate $pK_2'$ in urine and the experimental values have been compared with those for phosphate solutions of similar ionic strength. The data suggest that under the conditions of the present experiments the behavior of phosphate in urine conforms closely to that predicted for simple phosphate solutions by the Debye-Hückel equation. It has further been demonstrated that the $pK_2'$ of phosphate in urine may vary significantly and that under some circumstances failure to consider this variation may introduce appreciable errors into either the measurement or calculation of urinary "titratable acid."

## THEORETICAL CONSIDERATIONS

The dissociation constant of a weak acid may be determined with reasonable accuracy for dilute solutions by employing concentrations in the mass law equation. Thus, the value of K in the equation

$$(1) \qquad K = \frac{[H^+][A^-]}{[HA]}$$

remains constant over a wide range of concentrations. In the case of strong electrolytes or the salts of weak acids, the dissociation constant is not accurately predicted by the mass law equation if concentrations are employed. According to current theory, this deviation from ideal behavior is not the result of incomplete dissociation but is accounted for by changes in activities induced chiefly by interionic electrostatic forces. The variation from ideal behavior is expressed by the activity coefficient, $\gamma$:

$$(2) \qquad \gamma = \frac{a}{c},$$

where a is the observed activity and c, the concentration. In general, $\gamma$ decreases with increase in concentration and approaches unity at high dilution. If $\gamma \cdot c$ is substituted in the mass law equation, the change in K can then be predicted. Thus for the reaction $H_2PO_4^- \rightleftarrows H^+ + HPO_4^=$, the following equation may be written:

$$(3) \qquad K_2 = \frac{a_{H^+} \cdot \gamma_2 \cdot [HPO_4^=]}{\gamma_1 \cdot [H_2PO_4^-]}.$$

In the logarithmic form, this becomes:

$$(4) \quad pK_2 = pH + \log \frac{[H_2PO_4^-]}{[HPO_4^=]} + \log \frac{\gamma_1}{\gamma_2}$$

or:

$$(5) \quad pK_2 - \log \frac{\gamma_1}{\gamma_2} = pH + \log \frac{[H_2PO_4^-]}{[HPO_4^=]} = pK_2'.$$

$pK_2$ is the theoretical value for an infinitely dilute solution where $\gamma_1$ and $\gamma_2 = 1$. At any finite

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concentration, however, the observed pK ($pK_2'$) will vary from $pK_2$ by the term $\log \frac{\gamma_1}{\gamma_2}$.

The relationship between the activity coefficient of an ion and ionic strength, $\mu$, of the solution may be expressed by the Debye-Hückel equation:

$$(6) \qquad -\log \gamma_i = \frac{A \cdot Z_i^2 \sqrt{\mu}}{1 + B\, a \sqrt{\mu}}.$$

$Z_i$ is the valence of the ion. A and B are constants, dependent upon temperature and the solvent. a is the "interionic diameter." The ionic strength of the solution, $\mu$, is calculated by multiplying the concentration of each ion, $C_i$, by the square of its valence $Z_i^2$ adding all such products, and dividing by 2. $\mu = \frac{\Sigma\, C_i\, Z_i^2}{2}$.

Substitution of 4.5 Å for a and the appropriate values for A and B (1) in Equation (6) yields an equation for each form of phosphate. The combination of these equations gives:

$$(7) \qquad \log \frac{\gamma_1}{\gamma_2} = \frac{1.57\sqrt{\mu}}{1 + 1.49\sqrt{\mu}}.$$

Equation (5) may then be rewritten:

$$(8) \qquad pK_2 - \frac{1.57\sqrt{\mu}}{1 + 1.49\sqrt{\mu}} = pH + \log \frac{[H_2PO_4^-]}{[HPO_4^=]} = pK_2'.$$

In this form, the equation is valid to an ionic strength of about 0.1. For ionic strengths above this, an additional term must be added which has been found to be linear with respect to ionic strength (2). The expanded equation may be written:

$$(9) \qquad pK_2 - \frac{1.57\sqrt{\mu}}{1 + 1.49\sqrt{\mu}} + \beta\mu = pH + \log \frac{[H_2PO_4^-]}{[HPO_4^=]} = pK_2'.$$

The value of $pK_2$ for phosphate solutions at 37° C., obtained by extrapolation to zero ionic strength, is approximately 7.181 (1). Thus if the ionic strength and ratio of phosphate are known and pH determined experimentally, the equation may be solved for $\beta$. The value of the linear constant, $\beta$, in the case of phosphate solutions is a function of the ratio of the two forms of phosphate (3). Bates and Acree (1) have studied the second dissociation constant of sodium phosphate solutions in which the fractions of total phosphate as $HPO_4^=$ were approximately 0.4, 0.5 and 0.6. These workers employed molal concentrations and measured E.M.F., using hydrogen and silver chloride electrodes and a technique which eliminated errors due to liquid junction potential. Since the method commonly employed in biologic work is pH measurement with the glass electrode, these constants have been restudied for sodium phosphate solutions at 37° C. using the glass electrode. Furthermore, molar concentrations have been employed in the present study in order to obtain results which could be compared more closely with those of urine where concentrations are ordinarily measured and expressed on a molar basis. In addition, linear constants have been determined for a wider range of phosphate ratios than studied by Bates and Acree, in order to encompass the range found in urine. No attempt was made to correct for liquid junction potential error in the pH measurement of either phosphate solutions or urine.

Within certain limits, the Debye-Hückel equation also predicts changes in activity coefficients for solutions containing more than one electrolyte. In dilute solutions, the activity coefficient of a given strong electrolyte is the same in all solutions of the same ionic strength (4). Thus in an equimolar phosphate buffer solution whose ionic strength is increased from 0.1 to 0.2 by the addition of sodium chloride, the change in activity coefficient is simply that predicted by the change in ionic strength (2). The effects of higher concentrations of NaCl have not previously been studied, nor have data been available on the influence of NaCl or other salts when phosphate is present in other than equimolar concentrations.

## METHODS

### *A. Experimental determination of $pK_2'$ phosphate and linear constants in simple solutions*

*1. Sodium phosphate solutions.* Standard solutions were made up so that the ratio of $H_2PO_4^-$ to $HPO_4^=$ was varied over a range from 19/1 to 0.43/1. For convenience, these ratios may be expressed as fractions of total phosphate present as $HPO_4^=$ and will be referred to as mole fractions in the remainder of this paper. The mole fractions, corre-

sponding to the above ratios, ranged from 0.05 to 0.70 and were achieved by individual weighings of $Na_2HPO_4$ and $NaH_2PO_4$. All solutions were made up in triplicate to a final molarity of 0.50.

The following procedure was carried out in duplicate with each standard solution: Successive 1 ml. aliquots were added from a buret to a beaker containing 10 ml. of distilled $H_2O$. By this means the molarity of the solution in the beaker was increased in a step-wise fashion from 0.0455 to 0.2500. The pH was determined after each increment. Substitution of pH and the appropriate values for $\mu$ and $\log \frac{[H_2PO_4^-]}{[HPO_4^=]}$ in Equation (*9*) was then made and the equation solved for $\beta$.

*2. Sodium phosphate solutions with added salts.* The effects of increasing quantities of NaCl and $Na_2SO_4$ on the $pK_2'$ of phosphate was studied by utilizing 50 and 100 mM sodium phosphate solutions of mole fractions, again ranging between 0.05 and 0.70. NaCl or $Na_2SO_4$ was added to a portion of each phosphate solution in quantities sufficient to make the final concentrations of these two salts 0.3 and 0.2 M, respectively. Successive 1 ml. aliquots of these latter solutions were then added from a buret to a beaker containing the comparable phosphate solution unmodified by addition of chloride or sulfate. In this way, the concentration and ratio of the two forms of phosphate was held constant while the concentration of the salt being studied was progressively increased. pH was measured after each 1 ml. increment and, from appropriate substitutions in Equation (*9*), $\beta$ was calculated.

### *B. Experimental determination of $pK_2'$ phosphate in urine*

Twelve experiments were performed on 11 female dogs anesthetized with 1 per cent chloralose. Urine composition was varied by infusion of sodium phosphate solutions (4 to 1 ratio of $HPO_4^=$ to $H_2PO_4^-$) at different rates or by addition of NaCl or $Na_2SO_4$ to the infusing solution. When phosphate alone was used, the infusate was brought to isotonicity by addition of glucose.

Since pH = $pK_2'$ when $[H_2PO_4^-] = [HPO_4^=]$, $pK_2'$ may be determined by measurement of pH when equimolar concentrations of phosphate have been established. This goal can be achieved experimentally if essentially all phosphate is first converted to $H_2PO_4^-$ by addition of acid and one-half of the phosphate then titrated to $HPO_4^=$ by addition of alkali. At a pH of 4.4 approximately 99 per cent of phosphate is present as $H_2PO_4^-$ over the widest range of $pK_1'$ and $pK_2'$ values which might be expected to occur in urine. If this pH lay exactly half-way between $pK_1'$ and $pK_2'$, the concentrations of $H_3PO_4$ and $HPO_4^=$ would be equal and therefore equivalent in buffer capacity to an equimolar quantity of $H_2PO_4^-$. However, the error introduced by ignoring the deviation from this ideal relationship in the subsequent determination of $pK_2'$ is, by calculation, less than 0.01.

The following experimental procedure was employed: Aliquots of each urine specimen were acidified to a pH of 4.4 ± 0.1 with 1.0 N HCl. One-tenth N NaOH was then added in an amount equivalent to one-half of the total phosphate. An additional amount of alkali was added equivalent to organic buffer present between approximately pH 4.4 and 6.8. The pH after addition of alkali was taken as the value of $pK_2'$.[1]

The ionic strength of urine specimens after addition of alkali was calculated by correcting the original electrolyte concentrations for the change resulting from the addition of HCl and NaOH. $H_2PO_4^-$ and $HPO_4^=$ were assumed to be present in equimolar concentrations and organic anions present between pH 2.7 and 6.8 were included in the calculation and taken as univalent.

### *C. Chemical measurements*

pH determinations were made with a Cambridge research model pH meter, using an external glass electrode and calomel reference electrode. All determinations were carried out in water bath maintained at 37 ± 1° C. The pH meter was standardized with National Bureau of Standards potassium acid phthalate (pH 4.025 at 37° C.), and the response of the entire assembly tested frequently by use of National Bureau of Standards sodium borate (pH 9.086 at 37° C.). Slight deviations from ideal response over this pH range were compensated for by adjustment of the temperature control mechanism (5). pH readings were made to the nearest 0.005 unit.

The following measurements were made on the urine by methods previously described (6): pH (anaerobic), sodium, potassium, ammonium, phosphate, chloride and $CO_2$ content. Inorganic sulfate was determined gravimetrically by the method of Folin (7). The sum of calcium and magnesium was measured by the method of Natelson and Penniall (8). Urines were prepared for organic acid titration by the method of Van Slyke and Palmer (9). In order to insure complete dephosphorylation, specimens were agitated for 30 minutes in an automatic wrist-action shaking device. The filtrates were acidified with 1 N HCl to pH 2.7 and titrated with 0.1 N NaOH. Titration readings were taken at pH 4.4, 6.8 and at the pH of the urine as excreted.

## RESULTS

### *A. $pK_2'$ and linear constants for sodium phosphate solutions*

Tables I and II present the data for phosphate solutions in which the mole fractions were 0.5 and 0.1, respectively. As shown in Column 1 of Table I, the solutions ranged in molarity from 0.0455 to 0.2500. Columns 2 and 3 give the ionic strength and square root of ionic strength, respectively, and Column 4 presents theoretical values for $pK_2'$ calculated from Equation (*8*). Each pH value appearing in Column 5 represents

[1] The same titration technique was applied to phosphate solutions of known ionic strength for the purpose of determining the accuracy of the method in simple solutions. Calculated and observed $pK_2'$ values agreed within ±0.02 unit.

TABLE I

*Experimentally determined $pK_2'$ values and derived linear constants, $\beta$, for sodium phosphate solutions containing equimolar concentrations of $H_2PO_4^-$ and $HPO_4^=$*

| Molarity | $\mu$ | $\sqrt{\mu}$ | $7.181 - \frac{1.57\sqrt{\mu}}{1+1.49\sqrt{\mu}}$ | pH ($pK_2'$) | $\beta\mu$ | $\beta$ |
|---|---|---|---|---|---|---|
| 0.0455 | 0.0910 | 0.302 | 6.854 | 6.848 | −0.006 | −0.07 |
| 0.0833 | 0.1666 | 0.408 | 6.782 | 6.753 | −0.029 | −0.17 |
| 0.1154 | 0.2308 | 0.480 | 6.741 | 6.714 | −0.027 | −0.12 |
| 0.1429 | 0.2858 | 0.535 | 6.714 | 6.672 | −0.042 | −0.15 |
| 0.1667 | 0.3334 | 0.577 | 6.694 | 6.656 | −0.038 | −0.11 |
| 0.1875 | 0.3750 | 0.612 | 6.678 | 6.632 | −0.046 | −0.12 |
| 0.2059 | 0.4118 | 0.642 | 6.666 | 6.619 | −0.047 | −0.11 |
| 0.2222 | 0.4444 | 0.667 | 6.656 | 6.605 | −0.051 | −0.11 |
| 0.2368 | 0.4736 | 0.688 | 6.648 | 6.596 | −0.052 | −0.11 |
| 0.2500 | 0.500 | 0.707 | 6.640 | 6.575 | −0.065 | −0.13 |

the average of six determinations. In Table I these values are taken to be the experimentally determined $pK_2'$ values, since $HPO_4^=$ and $H_2PO_4^-$ were present in equimolar concentrations. It can be seen that the experimentally determined $pK_2'$ values differ from those calculated from Equation (*8*) by an amount which is a linear function of ionic strength [Equation (*9*)]. This difference, $\beta\mu$, is shown in Column 6 and the linear constant, $\beta$, in Column 7. The $pK_2'$ values for this buffer ratio decreased from 6.848 to 6.575 as the ionic strength was increased from 0.091 to 0.500. $\beta$, however, remained relatively constant with an average value of −0.12.

Table II is similar to Table I in all respects except that $pK_2'$ was determined by adding the logarithm of the phosphate ratio to the measured pH value. The difference between these $pK_2'$ values and those calculated from Equation (*8*) are shown in Column 7 and the corresponding linear constant, $\beta$, in Column 8. $pK_2'$ fell progressively from 6.877 to 6.570 over a range of ionic strength from 0.0546 to 0.300. $\beta$ remained relatively constant and had an average value of −0.45.

In Figure 1 are shown the $\beta$ values for all mole fractions studied. The best fitting curve has been drawn through the points by inspection. The values for $\beta$ ranged from −0.51 for mole fraction 0.05 to −0.02 for mole fraction 0.7. It is apparent that the value rapidly becomes more negative as the ratio of acid to basic phosphate is increased.

By substitution of each value for $\beta$ in Equation (*9*) a curve describing the relationship between $pK_2'$ and ionic strength may be obtained for each mole fraction. Figure 2 shows the family of curves derived by this method. At low ionic strengths (less than 0.04) there is no appreciable difference in $pK_2'$ for various mole fractions. However, as ionic strength is increased, the curves diverge. Thus at an ionic strength of 0.36, for example, $pK_2'$ lies between 6.51 and 6.67.

TABLE II

*Experimentally determined $pK_2'$ values and derived linear constants, $\beta$, for sodium phosphate solutions containing a nine to one ratio of $H_2PO_4^-$ to $HPO_4^=$*

| Molarity | $\mu$ | $\sqrt{\mu}$ | $7.181 - \frac{1.57\sqrt{\mu}}{1+1.49\sqrt{\mu}}$ | pH | pH + log R ($pK_2'$) | $\beta\mu$ | $\beta$ |
|---|---|---|---|---|---|---|---|
| 0.0455 | 0.0546 | 0.234 | 6.909 | 5.923 | 6.877 | −0.032 | −0.59 |
| 0.0833 | 0.0999 | 0.316 | 6.844 | 5.852 | 6.806 | −0.038 | −0.38 |
| 0.1154 | 0.1385 | 0.372 | 6.805 | 5.799 | 6.753 | −0.052 | −0.38 |
| 0.1429 | 0.1715 | 0.414 | 6.779 | 5.755 | 6.709 | −0.070 | −0.41 |
| 0.1667 | 0.2001 | 0.447 | 6.760 | 5.713 | 6.667 | −0.093 | −0.46 |
| 0.1875 | 0.2251 | 0.474 | 6.745 | 5.682 | 6.636 | −0.109 | −0.48 |
| 0.2059 | 0.2471 | 0.497 | 6.733 | 5.667 | 6.621 | −0.112 | −0.45 |
| 0.2222 | 0.2666 | 0.516 | 6.723 | 5.645 | 6.599 | −0.124 | −0.47 |
| 0.2368 | 0.2842 | 0.533 | 6.714 | 5.627 | 6.581 | −0.133 | −0.47 |
| 0.2500 | 0.300 | 0.548 | 6.708 | 5.616 | 6.570 | −0.138 | −0.46 |

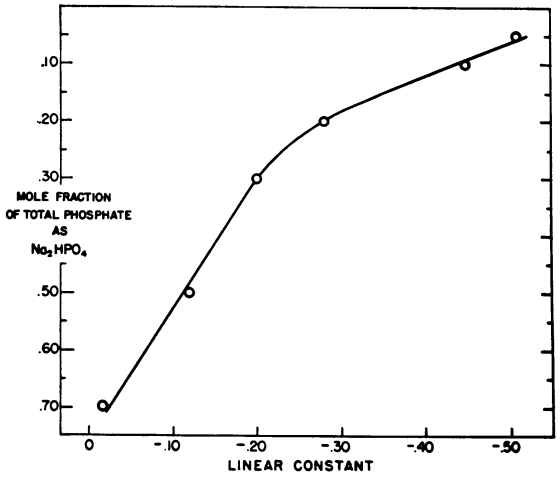


FIG. 1. RELATIONSHIP BETWEEN THE LINEAR CONSTANT, $\beta$, AND THE RATIO OF $HPO_4^=$ TO $H_2PO_4^-$

The addition of NaCl in quantities up to 100 mM per L. had no significant effect on the $\beta$ values of 50 and 100 mM phosphate solutions over the entire range of mole fractions (0.05 to 0.70). At higher concentrations of NaCl, the value for $\beta$ rose progressively, and thus $\beta$ cannot be expressed as a single value at NaCl concentrations above 100 mM per L.

$Na_2SO_4$ in concentrations up to 100 mM per L. had no effect on the $\beta$ value of equimolar phosphate solutions. In more acid phosphate solutions, however, $Na_2SO_4$ in concentrations from 20 to 100 mM per L. significantly affected the values for $\beta$, although the new values were essentially constant over this range of sulfate concentrations. For the mole fractions 0.05, 0.10, 0.20, 0.30 and 0.50 the values of $-\beta$ were 0.31, 0.25, 0.20, 0.17 and 0.12, respectively.

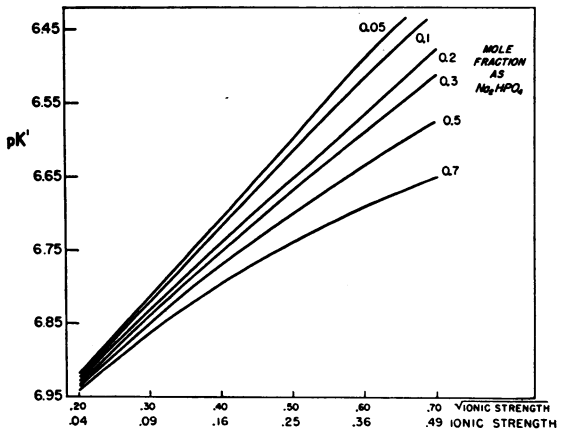


FIG. 2. RELATIONSHIP BETWEEN PHOSPHATE $pK_2'$ AND IONIC STRENGTH

Each curve represents the $pK_2'$ values for a given ratio of $HPO_4^=$ to $H_2PO_4^-$.

### *B. $pK_2'$ of phosphate in urine*

Table III shows the results of a typical experiment. The infusing solutions consisted of sodium phosphate plus glucose during Periods 1 through 4 and sodium phosphate plus saline during Periods 5 through 8. The electrolyte concentrations shown in the table have been corrected for the changes resulting from the titration procedure, and the ionic strength ($\mu$) calculated from these concentrations.[2] The last two

[2] The sum of calcium and magnesium was measured in representative urines from each experiment and was found to be approximately 1 mM per L. in most specimens and in no instance greater than 2 mM per L. In view of these low concentrations, the effects of complex ion formation with divalent anions was ignored in both the experimental determination of $pK_2'$ and the calculation of ionic strength. It is also recognized that the ionic strength calculated for

TABLE III

*Effect of variation in urinary ionic strength on $pK_2'$ phosphate during acute phosphate loading*

| Period | Na | K | $NH_4$ | Cl | $PO_4$ | Organic acid | $\mu$ | $\sqrt{\mu}$ | $pK_2'$ calculated | $pK_2'$ observed |
|---|---|---|---|---|---|---|---|---|---|---|
| | *mEq./L.* | *mEq./L.* | *mEq./L.* | *mEq./L.* | *mM/L.* | *mEq./L.* | | | | |
| | Infusion of $Na_2HPO_4$, 40 mM/L.; $NaH_2PO_4$, 10 mM/L.; glucose, 30 Gm./L. | | | | | | | | | |
| 1 | 55 | 27 | 18 | 7 | 44 | 27 | 0.1220 | 0.349 | 6.81 | 6.82 |
| 2 | 39 | 9 | 7 | 6 | 24 | 21 | 0.0710 | 0.266 | 6.88 | 6.91 |
| 3 | 39 | 5 | 5 | 10 | 19 | 19 | 0.0628 | 0.251 | 6.89 | 6.91 |
| 4 | 36 | 4 | 3 | 10 | 18 | 15 | 0.0565 | 0.238 | 6.90 | 6.91 |
| | Infusion of $Na_2HPO_4$, 40 mM/L.; $NaH_2PO_4$, 10 mM/L.; NaCl, 500 mM/L. | | | | | | | | | |
| 5 | 35 | 4 | 3 | 12 | 17 | 12 | 0.0543 | 0.233 | 6.90 | 6.91 |
| 6 | 61 | 6 | 4 | 25 | 26 | 7 | 0.0840 | 0.290 | 6.85 | 6.88 |
| 7 | 96 | 5 | 3 | 61 | 23 | 6 | 0.1143 | 0.338 | 6.81 | 6.79 |
| 8 | 138 | 5 | 2 | 100 | 23 | 6 | 0.1543 | 0.393 | 6.77 | 6.76 |

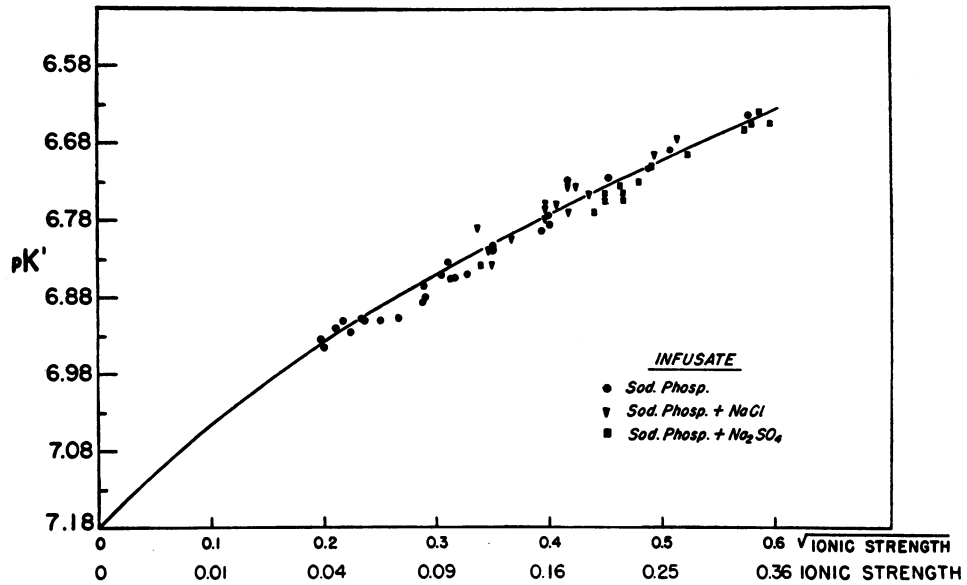


FIG. 3. EXPERIMENTALLY DETERMINED $pK_2'$ VALUES FOR PHOSPHATE IN URINE

The experimental points have been plotted as a function of the calculated ionic strength of the urine. The solid line describes the relationship between $pK_2'$ and ionic strength for equimolar sodium phosphate solutions and is described by Equation (*9*) with a $\beta$ value of $-0.12$.

columns compare calculated and experimentally observed $pK_2'$ values. These differed by $\pm 0.02$ unit or less in six observations and by 0.03 in the remaining two. During the course of the experiment, there was a threefold variation in ionic strength and $pK_2'$ varied between 6.91 and 6.76.

Figure 3 summarizes the results of all 12 experiments. The solid line describes the relationship between $pK_2'$ and ionic strength for phosphate solutions of 0.5 M fraction. The observed $pK_2'$ values have been plotted as a function of the estimated ionic strength of each specimen after titration. The range of observed values was from 6.95 to 6.64. The experimental points fell within $\pm 0.02$ pK unit of the predicted values in 80 per cent of determinations and within $\pm 0.03$ in the remainder.

The presence of chloride or sulfate up to a concentration of approximately 100 mM per L. had no apparent effect upon the agreement between observed and theoretical values.

urine is not exactly comparable to the ionic strength of a simple phosphate solution (when both calculations are based on molar concentrations) since substances other than phosphate present in urine alter the ratio of water to total solute. This slight discrepancy has also been ignored.

## DISCUSSION

The data indicate that, under the present experimental conditions, the $pK_2'$ of phosphate in urine changes as a function of ionic strength in a fashion similar to that observed in simple phosphate solutions and in accord with the predictions of Debye-Hückel theory [Equation (*9*)]. The experimentally determined $pK_2'$ values ranged from 6.64 to 6.95. As in equimolar sodium phosphate solutions, the $pK_2'$ was apparently uninfluenced by the presence of large amounts of chloride or sulfate, except to the extent that these ions contributed to the total urinary ionic strength.

The experimentally determined $pK_2'$ values cannot be taken as those of the original urines, since the titration procedure employed in the measurement of $pK_2'$ phosphate in itself changed the composition of the urine. The original values could be calculated if one knew both the initial urinary ionic strength and the phosphate ratio with its appropriate linear constant, $\beta$. However, $\beta$ and the calculation of ionic strength depend on the ratio of the two forms of phosphate, a proportion which cannot be determined analytically. This problem can be circumvented,

and a close estimate of $pK_2'$ obtained, by means of the following series of approximations:

1. The ratio of $HPO_4^=$ to $H_2PO_4^-$ is estimated from the Henderson-Hasselbalch equation, using the measured urine pH and an arbitrary $pK_2'$ value of 6.8.

2. The ionic strength of the urine is calculated from the individual electrolyte concentrations, the contribution of phosphate being estimated from the ratio calculated in Step 1.

3. An appropriate $\beta$ value is chosen from the phosphate ratio, and utilizing this and the ionic strength, a value for $pK_2'$ is calculated from Equation (*9*). In the choice of an appropriate linear constant the possible influence of any other ion present in high concentration must be considered. In the case of chloride, it has been demonstrated that concentrations up to 100 mM per L. exert no significant effect on the $\beta$ value of 50 and 100 mM sodium phosphate solutions over a wide range of buffer ratios. On the other hand, sulfate significantly modifies $\beta$ except when the two forms of phosphate are present in equimolar concentrations (see Results).

4. The entire process of calculation is repeated, using the $pK_2'$ value obtained in Step 3. The resulting $pK_2'$ ordinarily is found to vary by less than 0.01 from that derived in the first series of calculations.

This method of approximation, when applied to phosphate solutions of known composition, yields results within $\pm 0.01$ of the correct $pK_2'$. The $pK_2'$ phosphate values for the urine as excreted, when estimated by this method, were found to lie between 6.56 and 6.98.

The $pK_2'$ of phosphate in normal plasma, where ionic strength is estimated to be 0.167 (10), has generally been taken as approximately 6.8. Based on the behavior of phosphate in solutions where ionic strength, alkalinity and sodium chloride concentrations are comparable to plasma, this value appears to be reasonable. The variations in plasma ionic strength and pH ordinarily encountered *in vivo* would be expected to induce a shift in $pK_2'$ of only several hundredths. Plasma thus stands in contrast to urine where $pK_2'$ varies widely as a result of the much greater range in ionic strength and pH.

The variability of $pK_2'$ in urine indicates that the ratio of $HPO_4^=$ to $H_2PO_4^-$ may differ appreciably in plasma (glomerular filtrate) and urine even when both solutions are at the same pH. Thus in the measurement of "titratable acid," it cannot be assumed that returning the urine pH to that of the plasma will necessarily restore the phosphate buffer ratio to that which was filtered at the glomerulus. To the extent that the ratio in the urine after titration to plasma pH differs from the ratio which existed in the filtrate, the estimate of renal acid excretion will be in error. Table IV illustrates the magnitude of errors which would result from assuming a $pK_2'$ of 6.8 for urine if this value were actually 6.6. $pK_2'$ of phosphate in plasma (and filtrate) has been taken as 6.8. The errors have been calculated by comparing the "titratable acid" obtained by returning the phosphate ratio to that in the glomerular filtrate (true acid excretion) with "titratable acid" obtained by titrating the urine to plasma pH (usual method). In Section A the plasma pH has been taken as 7.4 and in Section B, 7.1. It is apparent that regardless of the initial urine pH, the quantity of phosphate over-titrated is the same and the absolute error is therefore constant. However, the

TABLE IV

*Errors in "Titratable Acid" (T.A.) resulting from use of 6.8 for $pK_2'$ phosphate when true value is 6.6* *

| Initial urine pH | Section A Blood pH, 7.4 | | | | Section B Blood pH, 7.1 | | | |
|---|---|---|---|---|---|---|---|---|
| | Apparent T.A. at pH 7.4 | True T.A. $\frac{HPO_4}{H_2PO_4} = \frac{4}{1}$ | Absolute error | Per cent error | Apparent T.A. at pH 7.1 | True T.A. $\frac{HPO_4}{H_2PO_4} = \frac{2}{1}$ | Absolute error | Per cent error |
| | *mEq./L.* | *mEq./L.* | *mEq./L.* | | *mEq./L.* | *mEq./L.* | *mEq./L.* | |
| 5.0 | 83.9 | 77.6 | +6.3 | + 8.1 | 73.6 | 64.2 | +9.4 | + 14.6 |
| 6.0 | 66.1 | 59.8 | +6.3 | +10.5 | 55.8 | 46.4 | +9.4 | + 20.3 |
| 6.4 | 47.7 | 41.4 | +6.3 | +15.2 | 37.4 | 28.0 | +9.4 | + 33.6 |
| 6.8 | 24.9 | 18.6 | +6.3 | +33.9 | 14.6 | 5.2 | +9.4 | +180.8 |

* Calculations based on urine phosphate concentrations of 100 mM per L.

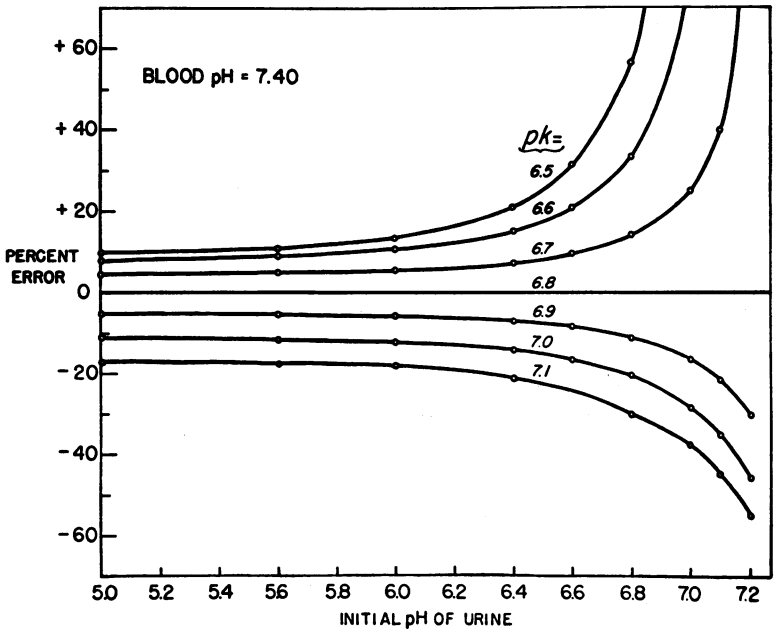


FIG. 4. PER CENT ERRORS IN "TITRATABLE ACID" RESULTING FROM USE OF AN ASSUMED $pK_2'$ PHOSPHATE OF 6.8 IN URINE RATHER THAN THE TRUE VALUE

On the abscissa are shown the initial pH values of the urine. Blood pH has been taken as 7.4.

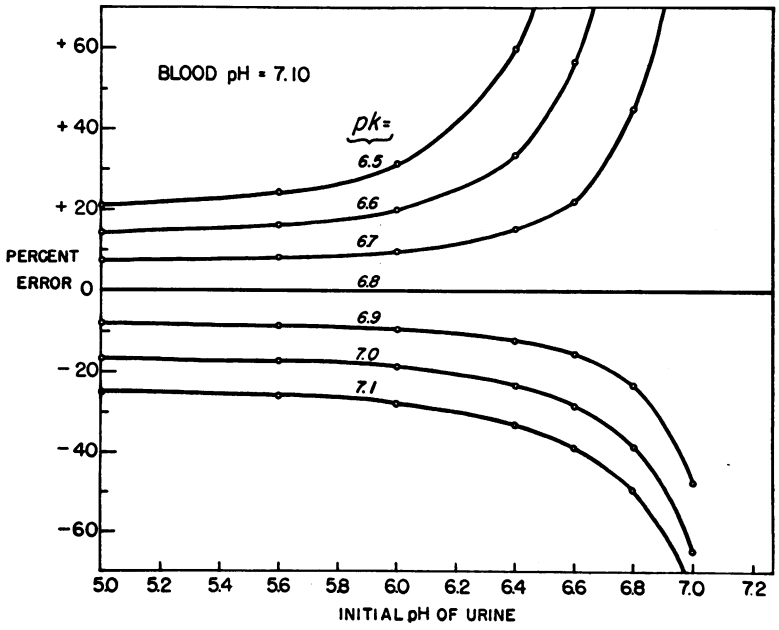


FIG. 5. PER CENT ERRORS IN "TITRATABLE ACID" RESULTING FROM USE OF AN ASSUMED $pK_2'$ PHOSPHATE OF 6.8 IN URINE RATHER THAN THE TRUE VALUE

On the abscissa are shown the initial pH values of the urine. Blood pH has been taken as 7.1.

per cent error is a function of total "titratable acid" and thus is greater with higher initial urine pH values. When plasma pH is lower than normal, the errors in "titratable acid" are larger. Thus, when the same urines are returned to a plasma pH of 7.1, the absolute error is greater and the per cent error for each starting pH is also significantly increased.

Figures 4 and 5 show the per cent errors in "titratable acid" which are introduced by assuming a $pK_2'$ of 6.8 when the true values range between 6.5 and 7.1. In Figure 4, the plasma pH has been taken as 7.4 and in Figure 5, as 7.1. On the abscissa is shown initial urine pH and on the ordinate the positive or negative error expressed as a per cent of the true "titratable acid." Each curve thus describes the errors for a given $pK_2'$ phosphate over a wide range of initial urine pH values. Between pH 5 and 6, the errors are relatively small but increase in a hyperbolic fashion between pH 6 and 7. As illustrated in Table IV, the errors are significantly larger when acidosis is present.

Regardless of the original $pK_2'$ of phosphate in the urine, dilution of the specimen prior to titration will elevate $pK_2'$ above the initial value. For example, a 1 : 5 dilution causes an upward shift in $pK_2'$ of 0.1 to 0.2 unit. In the case of urines of high ionic strength and low $pK_2'$ phosphate, dilution might fortuitously shift $pK_2'$ to a value near 6.8 and thus reduce the error in "titratable acid" which would have occurred if urine pH had arbitrarily been returned to that of plasma. However, a 1 : 5 dilution shifts any initial $pK_2'$ phosphate of 6.7 or above to values between 6.90 and 7.10 and a 1 : 10 dilution shifts $pK_2'$ phosphate of even the most concentrated specimens to this same range. The magnitude of the resulting errors in the measurement of "titratable acid" is shown in Figures 4 and 5.

Close agreement between measured "titratable acid" and a value calculated from the Henderson-Hasselbalch equation, utilizing a $pK_2'$ of 6.8, has often been observed during phosphate loading (11, 12). This agreement would have resulted if the urine $pK_2'$ phosphate values were, in fact, approximately 6.8. However, it can be readily shown that all phosphate buffer solutions with $pK_2'$ values between 6.6 and 7.0 have approximately the same buffer capacity when they are titrated from an initial pH between 5.6 and 6.6 to a final pH between 7.1 and 7.4. Thus, agreement with a calculated value for "titratable acid" may be observed when the ratio to which urinary phosphate has been returned actually differs significantly from that of the glomerular filtrate. This agreement is misleading since the true measure of acid excretion, as has been pointed out, requires restoration of the buffer ratio to that which existed in the filtrate.

It would thus appear that the contribution of phosphate to "titratable acid" can be estimated most accurately by calculation, utilizing the appropriate $pK_2'$ value. However, since $pK_2'$ of phosphate may be influenced by the presence of other electrolytes, a reliable estimate can be made only for urines in which the phosphate salt predominates or in which the effect of some other electrolyte present in high concentration is known. In 24 hour urine specimens, where many electrolytes may be present in high concentration, the situation is more complex and calculation of $pK_2'$ will be less dependable.

If the contribution of phosphate to "titratable acid" is calculated, the contribution of nonphosphate buffers can be determined by titration of dephosphorylated urine. The bulk of organic buffer ordinarily found in urine is comprised of substances whose pK' values are relatively low in comparison to the pH of plasma. Under these circumstances, no appreciable error will be introduced by ignoring variations in their dissociation constants and in arbitrarily titrating to the plasma pH.

## SUMMARY

1. The influence of urinary ionic strength on the apparent dissociation constant of phosphate, $pK_2'$, has been studied during phosphate loading experiments in dogs. $pK_2'$ was determined by measurement of pH at 37° C. after treatment of the urine by a method designed to establish equimolar concentrations of $HPO_4^=$ and $H_2PO_4^-$. The experimental $pK_2'$ values were found to lie between 6.64 and 6.95. Comparison of these $pK_2'$ values with those determined for equimolar sodium phosphate solutions of comparable ionic strength demonstrated agreement within $\pm 0.02$ unit in nearly all instances. The data indicate that, under the present experimental conditions, the behavior of phosphate in urine conformed closely to the predictions of Debye-Hückel theory.

2. The values for $pK_2'$ in the urine as originally excreted were estimated by a method of successive approximations and were found to lie between 6.56 and 6.98.

3. It is concluded that appreciable errors may occur in values of "titratable acid" obtained by calculation or by titration when a $pK_2'$ of 6.8 is arbitrarily assumed for the phosphate system in urine.

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