THE INFLUENCE OF ANION PENETRATING ABILITY ON
URINARY ACIDIFICATION AND THE EXCRETION
OF TITRATABLE ACID *

BY NORMAN BANK † AND WILLIAM B. SCHWARTZ ‡

(From the Department of Medicine, Tufts University School of Medicine, and the Pratt
Clinic-New England Center Hospital, Boston, Mass.)

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Previous studies in the dog have demonstrated that during administration of neutral sodium phos-
phate the rate of titratable acid excretion is directly propor-
tional to the rate of phosphate excretion and is inversely related to plasma bicarbonate con-
centration (1). Markedly acid urines containing near theoretical maximum amounts of titratable
acid 1 were observed only in the presence of se-
vere metabolic acidosis.

More recently it has been shown that intense acidification of the urine can be achieved without
the induction of acidosis (2). Normal subjects stimulated to retain sodium showed a rapid fall
in urine pH to approximately 5.0 or below when infused with sodium sulfate, and it was concluded
that increased reabsorption of sodium in the pres-
ence of a poorly reabsorbable anion was responsi-
ble for this phenomenon. Acidification of the urine has also been observed during administra-
tion of poorly reabsorbable anions to normal hu-
man subjects during quiet standing (3) and to
dogs in which glomerular filtration rate was re-
duced by clamping of the renal artery (4)—ex-
perimental procedures known to induce a more
complete reabsorption of filtered sodium. These
observations raise the possibility that, even without
acidosis, large numbers of hydrogen ions might be
transported into the glomerular filtrate over a

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Present address: Department of Medicine, New York
University Medical Center, New York, N. Y.

‡ Established Investigator of the American Heart
Association.

1 In urines where phosphate is the major buffer, values
closely approaching the theoretical maximum for titratable
acid are attained when urine pH falls to approximately
5.5, since at this point more than 95 per cent of the phos-
phate (pK, approximately 6.8) is in the H₂PO₄⁻ form.

comparably wide pH gradient if a poorly reabsorb-
able buffer anion were infused under appropriate
experimental conditions. In the present study it
has been demonstrated that, during the infusion
of sodium phosphate (pH 7.4) into dogs whose
sodium and chloride excretion had been reduced
by dietary salt restriction, excretion of titratable
acid rises to near theoretical maximum values in
the presence of normal plasma bicarbonate con-
centrations.

These data suggest the hypothesis that a
disproportion between the reabsorption of so-
dium and anion was a critical factor in regu-
lying not only the acidity of the urine but also
the rate of excretion of titratable acid. This hy-
pothesis has been further supported by the demon-
stration that administration of relatively reabsorb-
able anions, such as chloride or thiocyanate, to
phosphate-loaded dogs is associated with a pro-
gressive elevation of urine pH and reduction in
titratable acid excretion. On the other hand, ad-
ministration of poorly reabsorbable anions, such
as sulfate or ferrocyanide, is accompanied by sus-
tained or increased acidity of the urine and excre-
tion of near theoretical maximum amounts of
titratable acid.

As a possible mechanism to account for these
findings, it is proposed that the various anions af-
fect urine pH and acid excretion through their in-
fluence on the electrical gradient established by
active sodium transport across the renal tubular
cells. According to this hypothesis, the magnitude
of the gradient is determined by the penetrating
ability of the available anions, those which pene-
trate the tubular epithelium more readily having
a greater tendency to follow sodium and thus to
reduce the potential difference. Changes in urin-
ary acidity have tentatively been attributed to the
passive movement of hydrogen ions in response to
variations in the transtubular electrical gradient.
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Although it seems probable that active transport of hydrogen is also involved in the over-all acidifying process, the present data suggest that passive diffusion of hydrogen plays an important role in the mechanism governing urine acidification and the renal excretion of titratable acid.

METHODS

Twenty-one experiments were performed on 21 female mongrel dogs, varying in weight from 10 to 20 kg. The animals were fed 100 g of ground horse meat for 10 to 14 days prior to the day of the experiment. This diet contained approximately 4 mEq Na+, 8 mEq Cl−, and 6 mEq K+ daily. Sixty to 90 minutes before each experiment, 500 ml of water was administered through a gastric tube. Anesthesia was induced by intravenous administration of chloralose, 100 mg per kg, as a 1.5 per cent solution in 2 per cent dextrose. Additional amounts were administered during the course of the experiment as required.

Each experiment was begun with the intravenous infusion of 4 per cent dextrose in water at a rate of 2.4 to 6.0 ml per minute. After an adequate urine flow had been established (approximately 3 to 6 ml per minute), two control urine collections of 10 to 15 minutes each were obtained. The dextrose solution was then replaced by one containing 60 mmoles per L of sodium phosphate (48 mmoles HPO4=, 12 mmoles H2PO4−) delivered at 4.2 ml per minute. After collecting urine for four to seven periods of 10 to 15 minutes each, this solution was replaced by one of the following, in millimoles per liter: NaCl, 108 (9 experiments); Na2SO4, 54 (4 experiments); NaSCN, 108 (4 experiments); and Na4Fe(CN)6, 27 (4 experiments); each was delivered at 4.2 ml per minute. The concentration of sodium and its rate of administration was thus the same during sodium phosphate loading and during subsequent loading with other salts. In order to maintain the osmolarity of all solutions at about 220, appropriate amounts of dextrose were added to the Na₂SO₄ and Na₄Fe(CN)₆ solutions.

Urines were collected through an indwelling bladder catheter and the bladder was emptied by manual compression at the end of each period. pH was determined on aliquots collected anaerobically at the midpoint of each period. Heparinized arterial blood samples were collected for determination of pH, total CO₂, chloride and potassium.

Urine and blood pH were determined anaerobically at 37°C with a Cambridge model R pH meter. CO₂ contents were determined manometrically by the method of Peters and Van Slyke (5). Urine sodium and potassium were measured by a Baird flame photometer using an internal lithium standard. Urine phosphorus was determined by the method of Fiske and Subbarow (6). Chloride was measured in urine and plasma by titration with silver nitrate, using a potentiometric method (7). Urine ferrocyanide was measured by the method of Ber-liner, Kennedy and Hilton (8), ammonium by the method of Folin and Bell (9), and inorganic sulfate was determined gravimetrically as barium sulfate (5). Thiocyanate was measured in urine and plasma by the method of Barker (10). Chloride was calculated for specimens containing thiocyanate by subtracting the measured thiocyanate concentration from the sum of chloride and thiocyanate as determined by titration with AgNO₃.

In specimens containing ferrocyanide, chloride and phosphate were determined after removal of the ferrocyanide by precipitation with ZnSO₄.

The value for titratable acid was calculated from the urine phosphate, urine pH and blood pH. The pK₂ of phosphate in blood was taken to be 6.8. The same value was used arbitrarily for urine when urine pH was below 6.0, since the error introduced into the calculation of titratable acid is less than 5 per cent when pK₂ is as much as ±0.2 unit different from 6.8 (11). The pK₂ was calculated for individual specimens with pH values above 6.0, since in such urines appreciable errors in estimation of titratable acid may result from use of an incorrect pK₂. On these specimens additional analyses were made for CO₂ and organic acids (11) in order to make a closer approximation of ionic strength. The theoretical considerations and method of calculation of pK₂ from ionic strength have been described in a previous publication from this laboratory (11).

For each urine specimen, titratable acid has been expressed as a percentage of the theoretical maximum titratable acid. This latter value was calculated by subtracting from total phosphate excretion that amount already present as H₂PO₄− at the pH of the plasma (and filtrate). For example, when plasma pH is 7.4 and 20 per cent of the phosphate is thus present as H₂PO₄−, the value for theoretical maximum titratable acid is equivalent to 80 per cent of total urine phosphate.

RESULTS

A. Effect of sodium phosphate infusion on urine pH and titratable acid excretion. Tables I to IV show the results of four representative experiments. During the control periods urine pH ranged between 6.32 and 6.83, chloride excretion ranged between 8 and 27 μmoles per minute, and phosphate excretion was 6 μmoles per minute or less. With the infusion of sodium phosphate, phosphate excretion rose to between 149 and 186 μmoles per minute and titratable acid excretion to between 102 and 143 μEq per minute. In all but one of the experimental periods, titratable acid

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2 The contribution of organic acids to titratable acid excretion was found to be negligible by potentiometric titration of urine from which phosphate had been removed by calcium hydroxide precipitation. The titration was carried out between the original pH of the urine and the pH of the plasma.
ranged between 87 and 99 per cent of the theoretical maximum. Urine pH in each instance fell within 15 minutes to values approximately 0.5 to 1.0 unit below those of the control periods and, as phosphate excretion increased, fell further to a level which ranged between 4.43 and 5.80. Chloride excretion fell in each case from control levels to less than 10 μmoles per minute and sodium excretion rose progressively.

Blood pH ranged between 7.25 and 7.39 during control periods and from 7.26 to 7.35 during phosphate loading. Bicarbonate concentrations were between 18.5 and 24.2 mmoles per L during control periods and between 18.5 and 22.2 during phosphate loading; Pco₂ was between 37 and 51 mm Hg during control periods and between 37 and 47 during phosphate loading.

The results of 17 experiments in which sodium phosphate was infused initially and NaCl, Na₂SO₄ or Na₄Fe(CN)₆ was given subsequently, are shown in Figures 1 and 2. The periods of sodium phosphate infusion are shown in each figure as solid circles. The other symbols, referring to a latter portion of each study, will be discussed separately. Phosphate excretion is plotted on the abscissa and the percentage of theoretical maximum titratable acid on the ordinate. Two points shown for convenience at 210 μmoles per minute were actually 221 and 255. It is clear that, during infusion of sodium phosphate, titratable acid excretion was close to the theoretical maximum over the entire range of phosphate excretion. The absolute values for titratable acid excretion rose to a maximum of 195 μEq per minute during phosphate infusion. Urine pH during control periods was usually between 6.0 and 7.0 and with the infusion of sodium phosphate fell to levels between 4.4 and 6.0. The average chloride excretion during control periods was 10 μmoles per minute, with a range from 1 to 28. During phosphate loading chloride excretion fell to an average of 4 μmoles per minute, with a range of 1 to 12. There were usually small but significant increases in excretion of both potassium and ammonium during phosphate loading.

Plasma bicarbonate concentration was between 18 and 22 mmoles per L in almost all experiments, and blood pH ranged between 7.25 and 7.44. In the individual experiments, blood pH and total CO₂ changed only slightly during control and phosphate loading periods.

B. Effect of NaCl infusion on urine pH and titratable acid excretion. In Table 1 are shown the results of a typical experiment in which NaCl was given following a sodium phosphate infusion. With the administration of NaCl, titratable acid excretion fell from 99 per cent of theoretical maximum to 42 per cent. Urine pH rose abruptly from 4.71 to 6.41 and continued to rise to 6.80 as chloride was infused. Chloride excretion rose progressively. Phosphate excretion fell gradually.

### Table 1

**The effect of a sodium phosphate infusion and a subsequent infusion of sodium chloride (T.A.) excretion**

<table>
<thead>
<tr>
<th>Time</th>
<th>Na</th>
<th>K</th>
<th>NH₄</th>
<th>Cl</th>
<th>P</th>
<th>pH</th>
<th>pK⁺</th>
<th>T.A.</th>
<th>Theor. max. T.A.</th>
<th>Max. T.A.</th>
<th>Plasma pH</th>
<th>HCO₃⁻</th>
<th>Pco₂</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>μmoles/min</td>
<td>μEq/min</td>
<td>%</td>
<td>mmoles/L</td>
<td>mmHg</td>
<td>mEq/L</td>
<td></td>
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<tr>
<td>0</td>
<td>Infusion: dextrose, 222 mmoles/L at 4.2 ml/min</td>
<td>7.39</td>
<td>21.7</td>
<td>37</td>
<td>106</td>
<td></td>
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<td></td>
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<tr>
<td>50–65</td>
<td>11</td>
<td>28</td>
<td>29</td>
<td>12</td>
<td>5</td>
<td>6.32</td>
<td>7.34</td>
<td>21.5</td>
<td>41</td>
<td>95</td>
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<td></td>
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</tr>
<tr>
<td>65–80</td>
<td>43</td>
<td>36</td>
<td>28</td>
<td>27</td>
<td>5</td>
<td>6.79</td>
<td>7.35</td>
<td>20.4</td>
<td>38</td>
<td>93</td>
<td></td>
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<td>85</td>
<td>Infusion: Na₃HPO₄, 48; Na₂HPO₄, 12; dextrose, 110 mmoles/L; at 4.2 ml/min</td>
<td>7.33</td>
<td>22.2</td>
<td>43</td>
<td>93</td>
<td></td>
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<td></td>
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<tr>
<td>95–110</td>
<td>1</td>
<td>19</td>
<td>32</td>
<td>22</td>
<td>20</td>
<td>5.67</td>
<td>6.80</td>
<td>14</td>
<td>15.5</td>
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<td>110–125</td>
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<td>41</td>
<td>2</td>
<td>58</td>
<td>4.59</td>
<td>6.80</td>
<td>44</td>
<td>45</td>
<td>98</td>
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<td>125–140</td>
<td>43</td>
<td>59</td>
<td>45</td>
<td>3</td>
<td>109</td>
<td>4.43</td>
<td>6.80</td>
<td>84</td>
<td>85</td>
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<td>140–155</td>
<td>95</td>
<td>55</td>
<td>34</td>
<td>4</td>
<td>144</td>
<td>4.96</td>
<td>6.80</td>
<td>110</td>
<td>112</td>
<td>98</td>
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<td>155–170</td>
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<td>31</td>
<td>5</td>
<td>153</td>
<td>5.40</td>
<td>6.80</td>
<td>113</td>
<td>119</td>
<td>95</td>
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<td>170–185</td>
<td>117</td>
<td>48</td>
<td>32</td>
<td>5</td>
<td>157</td>
<td>5.42</td>
<td>6.80</td>
<td>116</td>
<td>122</td>
<td>95</td>
<td></td>
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<tr>
<td>185–200</td>
<td>141</td>
<td>63</td>
<td>36</td>
<td>5</td>
<td>186</td>
<td>4.71</td>
<td>6.80</td>
<td>143</td>
<td>144</td>
<td>99</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>202</td>
<td>Infusion: NaCl, 108 mmoles/L; at 4.2 ml/min</td>
<td>7.33</td>
<td>22.1</td>
<td>43</td>
<td>91</td>
<td></td>
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<td></td>
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<tr>
<td>215–230</td>
<td>181</td>
<td>37</td>
<td>24</td>
<td>15</td>
<td>149</td>
<td>6.41</td>
<td>6.84</td>
<td>76</td>
<td>116</td>
<td>66</td>
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<tr>
<td>230–245</td>
<td>212</td>
<td>42</td>
<td>22</td>
<td>32</td>
<td>124</td>
<td>6.60</td>
<td>6.86</td>
<td>52</td>
<td>96</td>
<td>54</td>
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<tr>
<td>245–260</td>
<td>184</td>
<td>38</td>
<td>21</td>
<td>26</td>
<td>101</td>
<td>6.60</td>
<td>6.87</td>
<td>40</td>
<td>78</td>
<td>51</td>
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<tr>
<td>260–275</td>
<td>173</td>
<td>33</td>
<td>21</td>
<td>28</td>
<td>80</td>
<td>6.80</td>
<td>6.88</td>
<td>26</td>
<td>62</td>
<td>42</td>
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The effect of sodium phosphate infusion and a subsequent infusion of sodium chloride on the percentage of theoretical maximum titratable acid excretion. For definition of "theoretical maximum titratable acid," see text.

during the next hour but the excretion rates were comparable with those of the preceding phosphate loading periods. Sodium excretion was higher than during the previous periods of phosphate loading.

The highest blood pH was 7.35, a value essentially the same as that during earlier periods when urine pH was between 4.43 and 5.67. Plasma bicarbonate rose about 1.5 mmoles per L and Pco₂ showed no significant change. Plasma chloride concentration rose 7 mEq per L.

The results of all nine experiments are shown in Figure 1 in which the NaCl periods are represented by open circles. The percentage of maximum titratable acid excretion fell progressively to levels well below those seen at comparable rates of phosphate excretion during sodium phosphate administration. Urine pH usually rose to above 6.0

### TABLE II

<table>
<thead>
<tr>
<th>Urine</th>
<th></th>
<th>Theor.</th>
<th>Max.</th>
<th>Plasma</th>
</tr>
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<tr>
<td></td>
<td>Time</td>
<td>Na</td>
<td>K</td>
<td>Cl</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>112</td>
<td>45</td>
<td>26</td>
</tr>
<tr>
<td>80-95</td>
<td>44</td>
<td>30</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>95-110</td>
<td>115</td>
<td>Infusion: dextrose, 222 mmoles/L, at 4.2 ml/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>125-140</td>
<td>6</td>
<td>43</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>140-150</td>
<td>18</td>
<td>69</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>150-160</td>
<td>36</td>
<td>77</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>160-170</td>
<td>63</td>
<td>79</td>
<td>36</td>
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</tr>
<tr>
<td>170-180</td>
<td>85</td>
<td>75</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>180-192</td>
<td>92</td>
<td>82</td>
<td>38</td>
<td>2</td>
</tr>
<tr>
<td>195</td>
<td>Infusion: Na₂HPO₄, 48; NaH₂PO₄, 12; dextrose, 55 mmoles/L; at 4.2 ml/min</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>205-215</td>
<td>139</td>
<td>80</td>
<td>39</td>
<td>4</td>
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<tr>
<td>215-225</td>
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<td>235-245</td>
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<td>245-255</td>
<td>218</td>
<td>76</td>
<td>41</td>
<td>3</td>
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</table>

**FIG. 1.** The effect of sodium phosphate infusion and a subsequent infusion of sodium chloride on the percentage of theoretical maximum titratable acid excretion.
shortly after NaCl was begun and continued to rise during the next 40 to 60 minutes to levels between 6.5 and 7.0 as chloride excretion increased from 15 to 130 μmoles per minute. In a few instances, a significant rise in urine pH and fall in titratable acid did not occur until the second period after beginning NaCl administration. Although it is not apparent from the figure, a close correlation existed between the first appearance of increased quantities of chloride in the urine and both the rise in pH and the fall in percentage of theoretical maximum titratable acid.\(^3\)

Blood pH during NaCl infusion ranged between 7.30 and 7.44, and plasma bicarbonate between 17 and 25 mmoles per L for the group. Changes in these values between the sodium phosphate and sodium chloride periods were small and variable.

C. Effect of Na\(_2\)SO\(_4\) infusion on urine pH and titratable acid excretion. In Table II are shown the results of a typical experiment in which Na\(_2\)SO\(_4\) was given following a sodium phosphate infusion. It is apparent that, during Na\(_2\)SO\(_4\) administration, titratable acid excretion remained at over 90 per cent of the theoretical maximum, and there was a slight further fall in urine pH. Chloride excretion was 2 to 3 μmoles per minute during phosphate administration and showed no significant change during sulfate infusion. Sodium excretion rose progressively throughout the entire experiment.

Blood pH, bicarbonate and chloride varied only slightly between the phosphate and sulfate infusion periods.

The results of all four experiments are shown in Figure 2, in which the open circles represent periods during Na\(_2\)SO\(_4\) infusion. The infusion of this salt was accompanied by excretion of near theoretical maximum amounts of titratable acid at rates of phosphate excretion comparable with those during sodium phosphate infusion. In three of the four experiments urine pH fell to lower levels than those achieved during phosphate infusion. In the fourth, there was no significant change from the acid values achieved during the phosphate periods. Chloride excretion was below 10 μmoles per minute during sulfate loading. Blood pH ranged between 7.30 and 7.40, plasma bicarbonate between

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\(^3\) In two experiments (not shown) initial acidification of the urine occurred with administration of phosphate but was followed by a sudden spontaneous rise in chloride excretion and pH, possibly due to an osmotic effect of the phosphate load. In a few additional experiments, where control chloride excretion was high (100 to 200 μEq per minute), little or no reduction in urine pH occurred during phosphate infusion.
ANION PENETRATING ABILITY AND URINARY ACIDIFICATION

TABLE III

The effect of a sodium phosphate infusion and subsequent infusions of sodium ferrocyanide and sodium chloride on urine pH and titratable acid excretion

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Urine</th>
<th>Plasma</th>
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<tbody>
<tr>
<td></td>
<td>Na(mmoles/min)</td>
<td>K(mmoles/min)</td>
</tr>
<tr>
<td>0</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>45-60</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>60-70</td>
<td>71</td>
<td>Infusion: Na₃HPO₄, 48; NaH₂PO₄, 12; dextrose, 55 mmoles/L; at 4.2 ml/min</td>
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<tr>
<td>80-90</td>
<td>90-100</td>
<td>115</td>
</tr>
<tr>
<td>100-110</td>
<td>100-110</td>
<td>149</td>
</tr>
<tr>
<td>110-120</td>
<td>110-120</td>
<td>174</td>
</tr>
<tr>
<td>122</td>
<td>Infusion: Na₄Fe(CN)₆, 27; dextrose, 83 mmoles/L; at 4.2 ml/min</td>
<td>7.31</td>
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<td>130-140</td>
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<td>172</td>
<td>Infusion: NaCl, 108 mmoles/L at 4.2 ml/min</td>
<td>7.31</td>
</tr>
<tr>
<td>180-190</td>
<td>180-190</td>
<td>445</td>
</tr>
<tr>
<td>190-200</td>
<td>190-200</td>
<td>413</td>
</tr>
<tr>
<td>200-210</td>
<td>200-210</td>
<td>308</td>
</tr>
<tr>
<td>210-220</td>
<td>210-220</td>
<td>289</td>
</tr>
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</table>

16 and 23 mmoles per L and Pco₂ between 30 and 40 mm Hg. These values were not significantly different from those of the preceding phosphate loading periods.

D. Effect of Na₄Fe(CN)₆ infusion on urine pH and titratable acid excretion. In Table III are shown the results of a typical experiment in which sodium ferrocyanide was given following a sodium phosphate infusion. The infusion of Na₄Fe(CN)₆ was accompanied by a slight rise in the percentage of theoretical maximum titratable acid to values of 98 to 99 per cent. Urine pH fell, from a range of 5.32 to 5.80, to 4.44 to 4.65, and chloride excretion remained below 10 μmoles per minute. During the last four periods, sodium ferrocyanide was replaced by sodium chloride. There was a prompt fall in the percentage of theoretical maximum titratable acid.⁴ Urine pH rose from 4.65 to 6.37 in the first period and continued to rise to 6.84. Ferrocyanide excretion continued in significant amounts and chloride excretion rose progressively. Sodium excretion increased during ferrocyanide infusion and decreased during the final periods of sodium chloride infusion.

Blood pH, plasma bicarbonate and Pco₂ remained essentially constant during the periods of both phosphate and ferrocyanide administration. Plasma chloride concentration was unchanged during ferrocyanide infusion and rose to 110 mEq per L when NaCl was administered.

The results of all four experiments are shown in Figure 2 in which the open squares represent the periods of Na₄Fe(CN)₆ infusion. It is evident that titratable acid excretion was more than 90 per cent of theoretical maximum at rates of phosphate excretion comparable with those during sodium phosphate infusion. Urine pH values in each instance were lower than those achieved during the previous phosphate infusion periods. Chloride excretion remained below 10 μmoles per minute during ferrocyanide infusion.

Blood pH ranged between 7.28 and 7.44, plasma bicarbonate between 17 and 22 mmoles per L and Pco₂ between 28 and 47 mm Hg during sodium ferrocyanide infusion. There were no significant changes from the values of the preceding phosphate loading periods.

E. Effect of NaSCN infusion on urine pH and titratable acid excretion. In Table IV are shown the results of the experiment in which occurred the most prompt response after starting sodium thiocyanate infusion. Urine pH rose from 5.74 to 6.62 and titratable acid excretion fell from 90

⁴ Since the specific effects of ferrocyanide on the pH of phosphate are not known, a value of 6.8 has been used arbitrarily.
TABLE IV  
The effect of a sodium phosphate infusion and a subsequent infusion of sodium thiocyanate on urine pH and titratable acid excretion

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Na (µmoles/min)</th>
<th>K (µmoles/min)</th>
<th>NH₄ (µmoles/min)</th>
<th>Cl (µmoles/min)</th>
<th>SCN (µmoles/min)</th>
<th>pH</th>
<th>pK₂'</th>
<th>Theor. T.A.</th>
<th>Max. T.A.</th>
<th>Plasma pH</th>
<th>HCO₃⁻</th>
<th>Pco₂</th>
<th>Cl</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td></td>
<td>222</td>
<td></td>
<td>4.2 ml/min</td>
<td></td>
<td>1</td>
<td>6.75</td>
<td></td>
<td></td>
<td>7.28</td>
<td>22.2</td>
<td>49</td>
<td>99</td>
</tr>
<tr>
<td>65–80</td>
<td>10</td>
<td>21</td>
<td>27</td>
<td>12</td>
<td></td>
<td>1</td>
<td>6.75</td>
<td></td>
<td></td>
<td>7.26</td>
<td>20.7</td>
<td>48</td>
<td>93</td>
</tr>
<tr>
<td>80–95</td>
<td>7</td>
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<td>28</td>
<td>8</td>
<td></td>
<td>1</td>
<td>6.60</td>
<td></td>
<td></td>
<td>7.26</td>
<td>20.3</td>
<td>47</td>
<td>91</td>
</tr>
<tr>
<td>120</td>
<td></td>
<td>33</td>
<td>36</td>
<td>38</td>
<td>8</td>
<td>53</td>
<td>6.16</td>
<td></td>
<td></td>
<td>7.26</td>
<td>20.3</td>
<td>47</td>
<td>91</td>
</tr>
<tr>
<td>130–140</td>
<td>26</td>
<td>24</td>
<td>24</td>
<td>2</td>
<td>50</td>
<td>6.80</td>
<td>6.80</td>
<td>87</td>
<td>97</td>
<td>90</td>
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<tr>
<td>140–155</td>
<td>104</td>
<td>44</td>
<td>28</td>
<td>4</td>
<td>130</td>
<td>6.80</td>
<td>6.80</td>
<td>98</td>
<td>108</td>
<td>91</td>
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<tr>
<td>170–185</td>
<td>122</td>
<td>48</td>
<td>36</td>
<td>4</td>
<td>146</td>
<td>6.80</td>
<td>6.80</td>
<td>102</td>
<td>114</td>
<td>90</td>
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<tr>
<td>200–215</td>
<td>144</td>
<td>49</td>
<td>33</td>
<td>5</td>
<td>149</td>
<td>6.74</td>
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<td>100</td>
<td>113</td>
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<tr>
<td>220</td>
<td></td>
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<td></td>
<td>4.2 ml/min</td>
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<td></td>
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<td></td>
<td>7.32</td>
<td>21.3</td>
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<td>8</td>
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<td>165</td>
<td>6.62</td>
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<td>126</td>
<td>48</td>
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<td>250–265</td>
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<td>1</td>
<td>105</td>
<td>6.67</td>
<td>6.87</td>
<td>100</td>
<td>80</td>
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<tr>
<td>265–275</td>
<td>210</td>
<td>42</td>
<td>23</td>
<td>22</td>
<td>2</td>
<td>107</td>
<td>6.79</td>
<td>6.91</td>
<td>110</td>
<td>85</td>
<td>43</td>
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</tr>
<tr>
<td>275–285</td>
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<td>59</td>
<td>28</td>
<td>70</td>
<td>5</td>
<td>107</td>
<td>6.85</td>
<td>6.90</td>
<td>102</td>
<td>82</td>
<td>38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To 48 per cent of theoretical maximum within 20 minutes. Thiocyanate excretion rose to 5 and chloride excretion from 5 to 70 µmoles per minute. A similar increase in chloride excretion following administration of thiocyanate has previously been noted by Rapoport and West (12).

Blood pH, plasma bicarbonate and Pco₂ remained within a normal range throughout the experiment.

In two of the other three experiments, there was a fall in titratable acid excretion from over 90 to less than 60 per cent of theoretical maximum and a progressive rise in urine pH from 5.56 and 5.28 to 6.58 and 6.57, respectively. However, these changes took place more slowly than in the experiments in which chloride was administered, and the maximal changes occurred only after 40 to 60 minutes of thiocyanate infusion. Furthermore, they were accompanied by only a slight rise in chloride and thiocyanate excretion. It is probable that this delay was due to almost complete reabsorption of thiocyanate and chloride proximal to the acidifying site. In the fourth experiment the changes in urine pH and titratable acid were small. At the end of 60 minutes pH had risen only from 5.44 to 5.98, and titratable acid excretion had fallen from 93 to 81 per cent of theoretical maximum. However, it should be noted that no thiocyanate appeared in the urine during this time, and there was no rise in chloride excretion.

In the latter three experiments, blood pH ranged between 7.25 and 7.35, plasma bicarbonate between 17 and 21 mmoles per L and Pco₂ between 34 and 49 mm Hg during NaSCN infusion. There were no changes from the values of the preceding sodium phosphate periods.

**DISCUSSION**

The present experiments demonstrate that administration of neutral sodium phosphate to dogs whose sodium chloride excretion has been reduced by dietary salt restriction is associated with a prompt and marked fall in urine pH and excretion of near theoretical maximum amounts of titratable acid. Titratable acid excretion remains close to these maximal values despite a progressive increase in phosphate excretion to as much as 250 µmoles per minute. The results indicate that, over the range of phosphate excretion rates which was studied, acidosis is not necessary for excretion of maximal amounts of acid. These observations are in accord with the view that a disproportion between sodium and anion reabsorption was the critical factor in determining not only the hydrogen ion gradient between blood and urine but also the total excretion of acid. This view is supported by the finding that the percentage of theoretical maximum titratable acid is decreased by the infusion of the readily reabsorbable anions, chloride and thiocyanate, and is sustained or increased by infusion of the poorly reabsorbable anions, sulfate or ferrocyanide.
No complete explanation for the influence of various anions on the process of acid excretion will be possible until more detailed information is available concerning the forces governing ion transfers across the renal tubule. However, it is of considerable interest that the results of the present experiments may readily be accounted for if it is assumed that the mechanism by which the anions affected acid excretion operated through their influence upon the electrical gradient across the renal tubular cells (13, 14). Active transport of sodium [which has recently been demonstrated for the renal tubule (15)] has been shown to account fully for the electrical potential difference (P.D.) across a variety of membranes (16, 17). The magnitude of the P.D. in such membranes depends upon both the rate at which sodium is transported and the resistance of the membrane to the ions that distribute themselves passively and which, by their diffusion, tend to reduce the P.D. (18, 19). In these terms the data in the present experiments might be explained in the following fashion: when sodium phosphate is infused into animals excreting little chloride, sodium reabsorption is increased at a time when only a relatively nonpenetrating anion, phosphate, is present in significant quantities. As a consequence, the P.D. across the tubular cell increases, the lumen becoming more negative, and H+ moves passively along the electrical gradient into the lumen, titrating the phosphate and reducing the urine pH.5 According to this hypothesis, not only phosphate but other anions in the filtrate should also influence the P.D. and, therefore, the acidifying process, in a manner dictated by their ability to penetrate the tubular epithelium. This "penetrating ability" has been shown by Rapoport and West (12) to follow closely the lyotropic series (SCN− > Cl− > HPO42− > SO42− > Fe(CN)63−) in a fashion similar to that observed for red cells, unicellular organisms, plant cells, and intestine (20, 21). Thus, when anions of low penetrating ability such as sulfate or ferrocyanide are infused, they sustain or increase the P.D. and the transfer of H+ to the filtrate. On the other hand, it is postulated that anions such as chloride or thiocyanate,6 which can move readily along the electrical gradient set up by active sodium transport, decrease the P.D. and reduce passive movement of hydrogen into the lumen even when large amounts of phosphate are present. This hypothesis is supported by the demonstration that the potential difference established across isolated frog skin by active sodium transport is much greater when a nonpenetrating anion such as sulfate is substituted in the bathing solution for a relatively penetrating anion such as chloride (19).

In the preceding discussion changes in pH and acid excretion have been described in terms of passive movement of hydrogen ions in response to a potential difference established across the renal tubule by active sodium transport. This view in no way excludes the possibility that active hydrogen transport plays an important role in the overall acidifying process but does suggest that anion penetrating ability can exert a critical influence on the mechanism governing urinary pH and the renal excretion of acid.

It is not meant to imply that the presence of large amounts of chloride in the urine is necessarily incompatible with aciduria. It is well known, for example, that during the chloruresis associated with ammonium chloride acidosis urine pH is low (23), and it seems probable that other factors such as a low intracellular pH, a reduced cellular potassium content, or a change in the permeability of the tubular epithelium to chloride might account for these findings. In this regard, it is of interest that permeability of the isolated frog skin to chloride may vary widely, e.g., when trace amounts of copper (10−5 M) are added to the bathing solution, chloride becomes virtually nonpenetrating (19). Under these circumstances potential differences of 120 to 130 mv develop across the membrane. Whether a comparable change in permeability as a consequence of metabolic acidosis might account for urinary acidification in the presence of chloruresis, or whether

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5 The simultaneous increase in potassium excretion observed in these experiments could also be accounted for by the movement of intracellular potassium into the filtrate in response to the increased negativity of the lumen.

6 It should be noted that thiocyanate inhibits acid secretion by the gastric mucosa without producing a significant change in electrical potential (22). However, in view of the striking differences between the mechanisms of gastric and renal acid formation, it seems improbable that the mode of action of thiocyanate on stomach and kidney is the same.
some other factors are responsible, remains to be determined.

SUMMARY

The influence on acid excretion of anions differing in penetrating ability has been studied in dogs whose sodium and chloride excretion had been reduced by dietary restriction. It was demonstrated that administration of the sodium salt of phosphate, a poorly reabsorbable buffer anion, leads to a marked reduction in urine pH and the excretion of near theoretical maximum amounts of titratable acid. These results indicate that, over the range of phosphate excretion rates which has been studied, maximal excretion of acid can be achieved without the stimulus of acidosis. It has been suggested that a disproportion between sodium and anion reabsorption was the critical factor in determining not only the pH gradient between blood and urine but also the total excretion of acid. This hypothesis is supported by the finding that the percentage of theoretical maximum titratable acid excreted in the urine is decreased by the subsequent infusion of the readily reabsorbable anions, chloride or thiocyanate, and is sustained or increased by the administration of the poorly reabsorbable anions, sulfate or ferrocyanide.

As a possible mechanism to account for these findings, it is proposed that the various anions affected urine pH and acid excretion through their influence on the electrical gradient established by active sodium transport across the renal tubular cells. According to this hypothesis, the magnitude of the gradient is determined by the relative penetrating ability of the available anions, those which penetrate the tubular epithelium more readily having a greater tendency to follow sodium and thus to reduce the potential difference. Changes in urinary pH and titratable acid excretion might thus be attributed to the passive movement of hydrogen ions in response to variations in the transtubular electrical gradient. This view in no way excludes the possibility that active hydrogen transport plays an important role in the over-all acidifying process but does suggest that anion penetrating ability can exert a critical influence on the mechanism governing urinary pH and the renal excretion of acid.

REFERENCES

1. Pitts, R. F., and Lotspeich, W. D. Factors governing the rate of excretion of titratable acid in the dog. Amer. J. Physiol. 1946, 147, 481.
ANION PENETRATING ABILITY AND URINARY ACIDIFICATION


