BICARBONATE AND THE RENAL REGULATION OF ACID BASE BALANCE

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Under the usual circumstance of ingestion of an acid ash diet the normal individual is faced with a deficit of available base. Those stores of circulating bicarbonate which enter the glomerular filtrate along with other crystalloids of the plasma are carefully conserved; only minute amounts are lost in the urine. But when an alkaline ash diet is ingested or sodium bicarbonate is administered, supplies of available base exceed the needs of the body, and the excess is excreted in the urine as bicarbonate. Reabsorption and excretion of bicarbonate constitute, therefore, the primary renal means of defending the body against depletion and expansion of its alkali reserve. The quantitative importance of the reabsorptive processes in the economy of the human body can be illustrated by a simple calculation. Approximately 190 liters of plasma, containing on an average 25 millimols of bicarbonate per liter, are filtered through the glomeruli in 24 hours (20). Thus, 4,750 millimols of bicarbonate, or 400 grams when expressed as the sodium salt, are tentatively excreted into the tubular urine each day, roughly 5 times the total body store of available base. A little over 2 millimols are excreted per day in 1.5 liters of urine of pH 6.0. Thus the reabsorptive mechanism is 99.95 per cent efficient under ordinary conditions. In contrast, the excretory processes are somewhat less impressive, although more than 100 grams of sodium bicarbonate have been ingested and excreted per day on certain therapeutic and experimental regimes.

The present paper is concerned with two aspects of the renal regulation of acid base balance: 1, a quantitation of the relationship between the plasma concentration and the rates of tubular reabsorption and urinary excretion of bicarbonate; 2, an elucidation of the relationship between the mechanism for the reabsorption of bicarbonate and the mechanism for acidifying the urine.

METHODS. Our experiments have been performed on normal female mongrel dogs trained to lie quietly with loose restraint on a comfortable animal board. The dogs were routinely fasted for 16 to 20 hours before use, and were well hydrated at the start of each experiment by the administration of approximately 40 cc. of water per kilo of body weight by stomach tube. In several experiments plasma bicarbonate was reduced by the oral administration of 200 to 300 cc. of 3 per cent ammonium chloride per day for several days prior to the experiment. In all experiments plasma bicarbonate was increased by the intravenous

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infusion of from 1 to 8 per cent sodium bicarbonate at a rate of 10 cc. per minute. The creatinine clearance was used as a measure of glomerular filtration rate.

Urine was collected with the following special precautions to prevent loss of carbon dioxide. A short silk retention catheter was introduced into the bladder and a rubber catheter of sufficient length to reach the bottom of a 250 cc. graduated cylinder was slipped over its free end. Urine was allowed to drain continuously into the cylinder beneath a heavy layer of paraffin oil. The bladder was emptied at the end of each collection period by manual compression of the lower abdomen; the rubber catheter was disconnected, and its contained urine was added to the specimen. Since urine flows were maintained at rates above 5 cc. per minute, fairly accurate collections were possible, although the bladder was not washed out. The urine specimen was mixed in the cylinder by gently stirring it with a baffle plate attached to a wire which dipped beneath the oil. Samples were withdrawn in pipettes for carbon dioxide content and pH determinations.

Special precautions were likewise taken to prevent loss of carbon dioxide during the collection and centrifugation of arterial blood samples. A retention needle fitted with a tight stylet was introduced into the femoral artery at the start of the experiment. Blood samples were drawn in 20 cc. oiled syringes containing 1 drop of neutral saturated potassium oxalate per 10 cc. of blood. A small amount of mercury served to mix blood and oxalate in the syringe. A portion of the blood was ejected through a spinal needle into a heavy walled centrifuge tube containing oil, the oil was displaced, and the completely filled tube was sealed with a rubber ampoule stopper. The blood was centrifuged immediately and stored in a refrigerator until the supernatant plasma could be analyzed for carbon dioxide (within 3 hrs.). Another portion of the blood was introduced into a 2 cc. Van Slyke stopcock pipette and stored on ice until pH measurement could be made. Since the pH of plasma and whole blood stored as long as 6 hours was identical with that of whole blood measured immediately, centrifuging and storing procedures were deemed adequate. A third portion of the blood was centrifuged without special precautions and plasma filtrates were prepared as previously described for the analysis of creatinine (14).

The carbon dioxide content of plasma and urine was determined by the method of Van Slyke and Neill (21). Analyses were performed on samples of urine varying from 5 cc. for acid urines to 0.2 cc. for the most alkaline urines. Plasma samples ranged from 1.0 to 0.2 cc. The pH of samples of urine and of whole blood was measured without exposure to air in a McInnes type glass electrode using a Cambridge pH meter. Measurements were made at room temperature (usually elevated to 30°C.) and corrected to 37°C. by subtracting 0.01 pH unit per degree difference in temperature (9). Bicarbonate and carbonic acid concentrations were calculated from carbon dioxide content and pH, using the Henderson-Hasselbalch equation and a pK' of 6.1 for both blood and urine. Carbonic acid concentrations were converted to carbon dioxide pressures using $\alpha = 0.0301$ for plasma, and $\alpha = 0.0309$ for urine according to Sendroy et al. (16).

The quantity of bicarbonate filtered through the glomeruli was calculated as the product of the plasma concentration and the glomerular filtration rate (creatinine clearance). The quantity excreted was calculated as the product
of the urine concentration and the urine flow. The difference between the 
quantity filtered and the quantity excreted is the quantity reabsorbed.

**RESULTS.** Reabsorption and excretion of bicarbonate as a function of plasma 
concentration. The relationship between the plasma concentration of bicarbonate 
and the quantity of bicarbonate filtered, excreted, and reabsorbed is illustrated 

**TABLE 1**

Experiments illustrating the relationship between the plasma concentration of bicarbonate and 
the quantities of bicarbonate filtered, excreted and reabsorbed in the dog. Experiment 1 
was performed in the fasting state, experiment 2 after feeding meat.

<table>
<thead>
<tr>
<th>TOTAL CONCURRENT TIME</th>
<th>GLOMERULAR FILTRATION RATE</th>
<th>ARTERIAL PLASMA CONCENTRATION</th>
<th>URINE CONCENTRATION</th>
<th>BICARBONATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
<td>Bicarbonate</td>
<td>Co2 pressure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cc./min.</td>
<td>mH. /l.</td>
<td>mm. /min.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mM.</td>
</tr>
<tr>
<td>min.</td>
<td>cc./min.</td>
<td>mmHg</td>
<td>cc./min.</td>
<td>mH. /l.</td>
</tr>
<tr>
<td>85</td>
<td>0.0% NaHCO3; 10 cc. per minute</td>
<td>7.50</td>
<td>0.08</td>
<td>22.0</td>
</tr>
<tr>
<td>105-115</td>
<td>7.95</td>
<td>10.9</td>
<td>25.9</td>
<td>4.4</td>
</tr>
<tr>
<td>115-125</td>
<td>7.94</td>
<td>10.2</td>
<td>23.9</td>
<td>8.2</td>
</tr>
<tr>
<td>125-135</td>
<td>8.23</td>
<td>14.8</td>
<td>28.9</td>
<td>9.2</td>
</tr>
<tr>
<td>145-155</td>
<td>7.41</td>
<td>18.3</td>
<td>20.9</td>
<td>6.7</td>
</tr>
<tr>
<td>155</td>
<td>8.43</td>
<td>23.5</td>
<td>23.3</td>
<td>1.44</td>
</tr>
<tr>
<td>165-175</td>
<td>7.55</td>
<td>29.4</td>
<td>34.8</td>
<td>11.8</td>
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<tr>
<td>175-185</td>
<td>7.61</td>
<td>34.4</td>
<td>25.5</td>
<td>13.6</td>
</tr>
<tr>
<td>185</td>
<td>9.68% NaHCO3; 10 cc. per minute</td>
<td>96.5</td>
<td>56.3</td>
<td>7.22</td>
</tr>
<tr>
<td>195-205</td>
<td>8.79</td>
<td>50.2</td>
<td>56.0</td>
<td>3.81</td>
</tr>
<tr>
<td>205-215</td>
<td>87.8</td>
<td>40.0</td>
<td>47.0</td>
<td>23.9</td>
</tr>
<tr>
<td>215-225</td>
<td>87.7</td>
<td>38.6</td>
<td>54.8</td>
<td>29.2</td>
</tr>
<tr>
<td>225-235</td>
<td>78.8</td>
<td>34.4</td>
<td>25.5</td>
<td>13.6</td>
</tr>
<tr>
<td>235-245</td>
<td>97.6</td>
<td>36.1</td>
<td>38.9</td>
<td>11.0</td>
</tr>
<tr>
<td>245-255</td>
<td>97.5</td>
<td>31.7</td>
<td>31.7</td>
<td>14.4</td>
</tr>
<tr>
<td>255-265</td>
<td>7.50</td>
<td>33.3</td>
<td>50.2</td>
<td>2.91</td>
</tr>
<tr>
<td>265-275</td>
<td>86.4</td>
<td>34.4</td>
<td>39.8</td>
<td>15.2</td>
</tr>
<tr>
<td>275-285</td>
<td>97.6</td>
<td>36.1</td>
<td>38.9</td>
<td>11.0</td>
</tr>
<tr>
<td>285-295</td>
<td>97.5</td>
<td>31.7</td>
<td>31.7</td>
<td>14.4</td>
</tr>
<tr>
<td>295-305</td>
<td>7.50</td>
<td>33.3</td>
<td>50.2</td>
<td>2.91</td>
</tr>
<tr>
<td>305-315</td>
<td>86.4</td>
<td>34.4</td>
<td>39.8</td>
<td>15.2</td>
</tr>
<tr>
<td>315-325</td>
<td>97.6</td>
<td>36.1</td>
<td>38.9</td>
<td>11.0</td>
</tr>
</tbody>
</table>

by experiment 1 in table 1. The low plasma concentration, evident in the first 
2 clearance periods of this experiment, resulted from the oral administration of 
200 cc of 3 per cent ammonium chloride per day for 6 days prior to the experi-
ment. Because of the low plasma bicarbonate, the quantity filtered through the 
glomeruli per unit of time was relatively small, and essentially all (99.95 per cent)
of that filtered was reabsorbed in its passage through the renal tubules. The urine contained negligible quantities of bicarbonate and was highly acid, pH 5.20. As the plasma concentration of bicarbonate was increased in stepwise fashion by the infusion of solutions of sodium bicarbonate of increasing concentration, the quantity filtered through the glomeruli increased in proportion. In the 3rd and 4th clearance periods as in the first two, essentially all the filtered bicarbonate was reabsorbed. However, when the quantity filtered exceeded 2.0 millimols per minute, frank excretion of bicarbonate occurred (periods 5 to 8). A limiting rate of reabsorption amounting to 1.8 to 1.9 millimols per minute was attained, which did not vary appreciably with plasma concentration. All of the bicarbonate filtered, above this limited quantity reabsorbed, was excreted in the urine.

Eighteen experiments similar to the one presented in table 1, comprising a total of 180 clearance periods, were performed on 4 dogs. These dogs varied considerably in functional renal capacity, their creatinine clearances ranging from 46 to 101 cc. per minute. To compare these animals upon a more or less uniform basis, reabsorptive and excretory capacities were calculated in terms of millimols of bicarbonate reabsorbed and excreted per 100 cc. of glomerular filtrate. Such values for experiment 1 are given in the last two columns of table 1. The data collected in all 18 experiments are plotted in figure 1. It is apparent from this figure that when the plasma concentration was below normal (10 to 20 millimols per liter) essentially all of the filtered bicarbonate was reabsorbed, and the quantity excreted was negligible. All animals behaved uniformly in this range of plasma concentration. In contrast, variability in completeness of reabsorption was noted in the different animals within the range of 20 to 25 millimols of bicarbonate per liter of plasma. Above 25 millimols per liter all animals excreted appreciable amounts of bicarbonate and the quantity excreted increased in linear proportion to the increase in plasma concentration. The average reabsorptive capacity above the threshold for frank excretion was 2.5 millimols per 100 cc. of glomerular filtrate. Dog 7 averaged somewhat lower than this mean value, dog 6 somewhat higher.

It is obvious from these data that the renal threshold for frank excretion approximates 25 millimols of bicarbonate per liter of plasma under the conditions of our experiments. Two factors must be considered in making specific applications of this experimental datum. The first factor is polyuria, necessitated in our experiments by our methods of urine collection. Urine pH usually rises moderately as urine flow increases, especially if the initial reaction is acid (1), and this increase in pH is accompanied by some slight elevation in the excretion of bicarbonate. Thus our diuretic animals might be expected to have somewhat lower renal thresholds than those with more usual urine flows. The second and more significant factor is that both the pattern and body store of electrolyte in our experimental animals were essentially normal except for the superimposed alterations in bicarbonate. In pathological states characterized by reduced plasma bicarbonate, it is usual to find a depletion of total body electrolyte, and common to find a disturbance of pattern as well (2, 12). We have observed that alterations in plasma chloride affect bicarbonate reabsorption even though the
Fig. 1. The renal reabsorption and excretion of bicarbonate in the dog as a function of plasma concentration. Note that the quantities reabsorbed and excreted are expressed in millimols per 100 cc. of glomerular filtrate.

chloride is administered as the sodium salt. When the plasma cation pattern is altered by the administration of potassium chloride, it is probable that even greater changes in reabsorptive capacity occur. Accordingly, in disease states
the renal bicarbonate threshold is subject to wider variations than those we have observed in our experimental animals.

Reabsorption of bicarbonate as a function of glomerular filtration rate. That a correlation exists between glomerular filtration rate and tubular reabsorptive capacity is implied by the data of figure 1. Such a correlation is to be expected in comparing animals of differing renal functional capacities for an obvious morphological correlation exists normally between the extent of the filtering surface of the glomeruli and the reabsorptive mass of the renal tubules. A question of more functional significance is whether or not in a given animal the tubular reab-

![Graph](http://ajplegacy.physiology.org/DownloadedFrom/10.220.33.3)
with periods at comparable plasma concentrations in experiment 2, it is apparent
that the increase in filtration rate was accompanied by an essentially equivalent
increase in reabsorptive capacity. Consequently reabsorptive capacity expressed
in millimols per 100 cc. of filtrate remained nearly the same.

In figure 2 the quantity of bicarbonate reabsorbed, expressed in millimols per
minute, is plotted against the glomerular filtration rate, including all clearance
periods from figure 1 in which the renal threshold for gross excretion had been
exceeded. The line represents the reabsorption of 2.5 millimols of bicarbonate
per 100 cc. of filtrate. From this graph it is evident that there is a direct correla-
tion between the tubular reabsorptive capacity and glomerular filtration rate in
animals with differing renal functional capacities. It is likewise evident in any
given animal that functional increases in filtration rate are accompanied by es-
sentially equivalent increases in reabsorptive capacity. This latter finding is of
significance in any interpretation of the reabsorptive limitation evident in figure
1. In the usual sense of the term, Tm or tubular maximum reabsorptive capacity,
cannot be applied to bicarbonate, for the tubular capacity to reabsorb, although
limited and independent of plasma concentration at any given filtration rate, is
nevertheless a direct function of filtration rate.

Interaction in the reabsorption of chloride and bicarbonate. The plasma concen-
trations of chloride and bicarbonate are normally maintained at their character-
istic levels by the independent excretion of that anionic component which is
present in excess. However, it has been repeatedly observed that total plasma
anion concentration tends to be maintained in the face of marked alterations in
anion pattern. Thus in alkalosis produced by pernicious vomiting, plasma
chloride is low and plasma bicarbonate correspondingly high (8). Conversely in
acidosis produced by diarrhea, plasma bicarbonate is low and plasma chloride
correspondingly high (7). It is reasonable to infer that the renal thresholds for
these two anions are interrelated in some fashion, the effect of which is to main-
tain the sum of their plasma concentrations within nearly normal limits. Experi-
ments 3 and 4 in table 2 indicate the extent of this interrelationship.

In experiment 3, the initial two clearance periods serve as controls. Plasma
bicarbonate and chloride concentrations were within a range of low normal
values, and urinary excretion of both bicarbonate and chloride was low. As a
consequence of the low bicarbonate excretion, the urine was moderately acid (pH
6.24). The infusion of sodium chloride (3 per cent in periods 3 to 5, 5 per cent in
periods 6 to 8 at 10 cc. per min.) progressively increased the plasma chloride con-
centration from 97 to 150 millimols per liter. Little change occurred in chloride
excretion until period 5. At a plasma concentration of 118 millimols per liter a
perceptible increase in excretion of chloride occurred, and accompanying this
increase was an augmented excretion of bicarbonate and a consequent elevation
of urinary pH. Effects are more striking in periods 6 to 8 in which the plasma
chloride concentration reached a value of 50 per cent above the initial control
value, and in which chloride and bicarbonate excretion rose correspondingly. It
is obvious from these data that the increased bicarbonate excretion resulted not

* Perhaps total cation concentration is the truc determining factor.
from any increase in the quantity filtered, but from a decrease in the quantity reabsorbed, i.e., from a lowering of the renal threshold.

Experiment 4 reversed the procedure of experiment 3. Elevation of plasma bicarbonate from low initial levels to levels within a range of normal (periods 5 and 6) was without effect on the excretion of either bicarbonate or chloride. However, at high plasma bicarbonate levels (periods 7 and 8), increased excretion of bicarbonate was accompanied by decreased reabsorption and increased excretion of chloride. It is apparent from these experiments that the capacity of the renal tubules to reabsorb bicarbonate is reduced by presenting to them simultaneously an excess of chloride. Similarly their capacity to reabsorb chloride is

### Table 2

Experiments illustrating the interrelationships of the renal thresholds for bicarbonate and chloride in the dog.

<table>
<thead>
<tr>
<th>TOTAL CONCURRENT TIME</th>
<th>GLOMERULAR FilTRATION RATE</th>
<th>ARTERIAL PLASMA CONCENTRATION</th>
<th>URINE FLOW</th>
<th>URINE CONCENTRATION</th>
<th>BICARBONATE</th>
<th>CHLORIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Filtered</td>
<td>Excreted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Filtered</td>
<td>Excreted</td>
</tr>
</tbody>
</table>

#### Experiment 3; dog 9

<table>
<thead>
<tr>
<th>min.</th>
<th>cc./ min.</th>
<th>mM./l.</th>
<th>cc./min.</th>
<th>mM./l.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>Filtered</th>
<th>Excreted</th>
<th>Re-absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Infuse: 0.0% NaCl; 10 cc per minute</td>
<td>58.8</td>
<td>22.7</td>
<td>95.4</td>
<td>8.1</td>
<td>6.34</td>
<td>1.44</td>
<td>3.00</td>
<td>1.33</td>
<td>0.011</td>
<td>1.32</td>
<td>5.60</td>
</tr>
<tr>
<td>120-130</td>
<td>55.3</td>
<td>22.3</td>
<td>97.2</td>
<td>8.4</td>
<td>6.34</td>
<td>1.37</td>
<td>2.60</td>
<td>1.24</td>
<td>0.012</td>
<td>1.23</td>
<td>8.4</td>
<td>0.022</td>
</tr>
<tr>
<td>141</td>
<td>Infuse: 3.0% NaCl; 10 cc per minute</td>
<td>61.4</td>
<td>21.0</td>
<td>103.</td>
<td>8.0</td>
<td>6.34</td>
<td>1.43</td>
<td>2.40</td>
<td>1.34</td>
<td>0.011</td>
<td>1.33</td>
<td>6.34</td>
</tr>
<tr>
<td>145-155</td>
<td>61.8</td>
<td>20.9</td>
<td>111.</td>
<td>7.1</td>
<td>6.31</td>
<td>1.63</td>
<td>3.40</td>
<td>1.29</td>
<td>0.011</td>
<td>1.28</td>
<td>6.88</td>
<td>0.024</td>
</tr>
<tr>
<td>155-165</td>
<td>63.7</td>
<td>21.0</td>
<td>118.</td>
<td>9.2</td>
<td>6.92</td>
<td>1.75</td>
<td>4.00</td>
<td>1.34</td>
<td>0.002</td>
<td>1.28</td>
<td>7.41</td>
<td>0.037</td>
</tr>
<tr>
<td>176</td>
<td>Infuse: 5.0% NaCl; 10 cc per minute</td>
<td>57.0</td>
<td>18.4</td>
<td>150.</td>
<td>10.4</td>
<td>7.28</td>
<td>16.0</td>
<td>173.</td>
<td>1.05</td>
<td>0.187</td>
<td>0.85</td>
<td>8.56</td>
</tr>
</tbody>
</table>

#### Experiment 4; dog 6

<table>
<thead>
<tr>
<th>min.</th>
<th>cc./ min.</th>
<th>mM./l.</th>
<th>cc./min.</th>
<th>mM./l.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>mM./ min.</th>
<th>Filtered</th>
<th>Excreted</th>
<th>Re-absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>Infuse: 0.0% NaHCO3; 10 cc per minute</td>
<td>56.8</td>
<td>12.7</td>
<td>108.</td>
<td>7.2</td>
<td>6.11</td>
<td>0.92</td>
<td>9.80</td>
<td>0.720</td>
<td>0.007</td>
<td>0.713</td>
<td>6.15</td>
</tr>
<tr>
<td>100-110</td>
<td>56.6</td>
<td>12.3</td>
<td>107.</td>
<td>6.7</td>
<td>6.25</td>
<td>1.18</td>
<td>6.40</td>
<td>0.695</td>
<td>0.008</td>
<td>0.687</td>
<td>6.06</td>
<td>0.043</td>
</tr>
<tr>
<td>110-120</td>
<td>58.9</td>
<td>14.5</td>
<td>109.</td>
<td>6.7</td>
<td>6.37</td>
<td>1.40</td>
<td>4.80</td>
<td>0.825</td>
<td>0.009</td>
<td>0.816</td>
<td>6.18</td>
<td>0.032</td>
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<tr>
<td>125-135</td>
<td>52.9</td>
<td>16.9</td>
<td>108.</td>
<td>7.2</td>
<td>6.37</td>
<td>1.53</td>
<td>6.00</td>
<td>0.695</td>
<td>0.011</td>
<td>0.684</td>
<td>5.71</td>
<td>0.043</td>
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<tr>
<td>135-145</td>
<td>54.7</td>
<td>23.5</td>
<td>105.</td>
<td>8.6</td>
<td>6.40</td>
<td>1.85</td>
<td>7.00</td>
<td>1.26</td>
<td>0.015</td>
<td>1.24</td>
<td>5.04</td>
<td>0.060</td>
</tr>
<tr>
<td>150-150</td>
<td>54.7</td>
<td>27.6</td>
<td>105.</td>
<td>10.0</td>
<td>6.65</td>
<td>3.70</td>
<td>4.00</td>
<td>1.51</td>
<td>0.037</td>
<td>1.47</td>
<td>5.04</td>
<td>0.040</td>
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<tr>
<td>155-165</td>
<td>62.1</td>
<td>37.7</td>
<td>105.</td>
<td>15.3</td>
<td>7.46</td>
<td>33.5</td>
<td>25.8</td>
<td>2.34</td>
<td>0.510</td>
<td>1.83</td>
<td>6.41</td>
<td>0.435</td>
</tr>
<tr>
<td>175-185</td>
<td>57.8</td>
<td>44.7</td>
<td>98.2</td>
<td>14.9</td>
<td>7.71</td>
<td>70.3</td>
<td>48.4</td>
<td>2.59</td>
<td>1.05</td>
<td>1.54</td>
<td>5.07</td>
<td>0.730</td>
</tr>
</tbody>
</table>
Reduced by presenting to them an excess of bicarbonate. Some of the variability in renal threshold and in bicarbonate reabsorptive capacity evident in the data of figures 1 and 2 may have been related to variations in plasma chloride concentration.

Fig. 3. The relationship between bicarbonate concentration and pH in 160 specimens of dog urine so collected as to prevent the loss of carbon dioxide. The smooth curve was calculated from the Henderson-Hasselbalch equation assuming a constant pCO₂ of 50 mm. Hg.

**Bicarbonate excretion and acid-base equilibria in urine.** Marshall (10) and Gamble (6) demonstrated that the concentration of bicarbonate in urines more acid than pH 6.0 is very low, and that it rises progressively in urines of increasing alkalinity, especially in those of pH 7.00 or above. They likewise demonstrated that excess body base is excreted almost entirely in the form of bicarbonate, which may increase to a value as high as 220 millimols per liter of urine. Since the
carbon dioxide pressure of the urine roughly approximates that of the blood (5), urines collected so as to avoid loss of carbon dioxide are never more alkaline than pH 8.0. Thus large quantities of base can be eliminated in urine which is only slightly more alkaline than blood.

In figure 3 the bicarbonate concentrations of 160 urine samples collected in 24 experiments are plotted against urine pH. The lowest urine pH was 4.96, the highest 7.96. The lowest bicarbonate concentration was 0.08 millimol per liter, the highest 197 millimols per liter. Between these extremes the relationship between bicarbonate concentration and pH was a uniform regular one and approximated that demanded by the Henderson-Hasselbach equation, assuming a constant carbon dioxide pressure of 50 mm. Hg (cf. smooth curve). However, the deviations from this theoretical curve are significant. Below pH 7.4 an assumed pressure of carbon dioxide of 50 mm. Hg is too high; above pH 7.6 it is too low. Thus the finding by Gamble (5) that urine pCO₂ is relatively constant over a wide range of urine pH is not borne out by our data. One obvious cause for this discrepancy is the very wide range of plasma bicarbonate concentration in our studies, 10 to 70 millimols per liter, which far exceeded the range in Gamble's human subjects. With such a range in plasma bicarbonate one would predict a wide range in plasma pCO₂, and if urine pCO₂ were related to plasma pCO₂, a similar range in urine pCO₂ would be expected.

In figure 4 the pCO₂ of the urine is plotted against the pCO₂ of the plasma, collected simultaneously. Urine pCO₂ varied from 22 mm. Hg to 109 mm. Hg, whereas plasma pCO₂ varied from 25 mm. Hg to 64 mm. Hg. Below a plasma pCO₂ of 40 mm. Hg there was relatively an even distribution of urine values above and below corresponding plasma values. Above a plasma pCO₂ of 40 mm. Hg, urine values tended to exceed plasma values. We fully recognize that our figures for pCO₂, since they are calculated, are subject to a number of sources of error such as variability of pK and a factors in different samples of blood and urine, random analytical errors, and the possibility of loss of small amounts of carbon dioxide in some samples. However, the following trends seem evident. 1. In acid urines, formed when plasma bicarbonate is subnormal, the urine pCO₂ tends to approximate the arterial plasma pCO₂. Carbon dioxide diffuses rapidly through most tissues, presumably through the renal epithelium as well. One would therefore predict that, within limits, equilibrium would be established between tubular urine, tubular cells, and renal venous blood with respect to carbon dioxide pressure. Since our plasma values are arterial this could mean that in acidosis the renal venous pCO₂ is essentially the same as the arterial pCO₂, i.e., the majority of the carbon dioxide produced in the oxidative metabolism of the kidney is added to the renal venous blood not as carbon dioxide, but as bicarbonate (cf. Pitts and Alexander (13, 15)). 2. On the other hand, in alkaline urines formed when plasma bicarbonate is above normal, the urine pCO₂ exceeds the arterial pCO₂ by a considerable margin and no doubt exceeds the renal venous pCO₂. This could well mean that a part of the reabsorption of bicarbonate occurs in that segment of the renal tubule which acidifies the urine by the exchange of intracellular H⁺ ion for Na⁺ ion of buffer salts present in the tubular urine (15).
Such an exchange involving bicarbonate would lead to the formation of carbonic acid in the tubular urine. Since the tubular urine contains no carbonic anhydrase, dehydration of carbonic acid to carbon dioxide and diffusion of carbon dioxide across the tubular epithelium into the renal venous blood might be sufficiently delayed to permit a significant elevation in urine pCO₂. Data presented below bear out this supposition.

The distal tubular reabsorption of bicarbonate and the mechanism for acidifying the urine. From a synthesis of the evidence obtained in the amphibian and mammalian kidney we infer that bicarbonate is reabsorbed by two dissimilar renal mechanisms located respectively in the proximal and distal tubules. In the frog and necturus the major part of the filtered bicarbonate is reabsorbed in the

Fig. 4. The relationship between the partial pressure of carbon dioxide in plasma and in urine collected simultaneously. The diagonal straight line indicates equivalence of partial pressure. The curve is the average of all points, fitted by inspection.
proximal segment of the renal tubules (22). Since the pH of the tubular urine at the end of the proximal segment is identical with that of the original filtrate, this reabsorptive process is an isohydric one (11), i.e., bicarbonate, carbon dioxide, base and water are reabsorbed in the same proportions in which they exist in the original filtrate. The remainder of the bicarbonate is ordinarily reabsorbed in the distal segment of the renal tubules, the process reaching completion at that level at which the urine attains its maximum acidity (11). It was suggested above that this final anisohydric reabsorption of bicarbonate is accomplished by that selfsame mechanism which, by exchanging H+ ions for Na+ ions of urinary buffer salts, acidifies the urine (13, 15). Two lines of experimental evidence support this view.

### TABLE 3

**Experiment illustrating the depression of the reabsorption of bicarbonate which is produced by the administration of sulfanilamide in the dog**

<table>
<thead>
<tr>
<th>TOT AL CONCURRE N T TIME</th>
<th>GL O MERUL AR PL ASMA CONCENTRATION</th>
<th>UR INE FLOW</th>
<th>UR INE CONC EN TRAT ION</th>
<th>BICARBONATE</th>
<th>CHLORIDE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>Bi-car bo nate</td>
<td>Chloride</td>
<td>Sul-fani lam ide</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>min.</td>
<td>cc./ min.</td>
<td>mM./ l.</td>
<td>mM./ l.</td>
<td>min.</td>
</tr>
<tr>
<td>90</td>
<td>Infuse: 0.0% sulfanilamide; 10 cc. per minute</td>
<td>52.0</td>
<td>7.44</td>
<td>22.5</td>
<td>101</td>
</tr>
<tr>
<td>115-125</td>
<td>55.6</td>
<td>7.44</td>
<td>22.0</td>
<td>101</td>
<td>0.0</td>
</tr>
<tr>
<td>125-145</td>
<td>54.2</td>
<td>7.44</td>
<td>22.0</td>
<td>101</td>
<td>0.0</td>
</tr>
<tr>
<td>140</td>
<td>Prime: 200 cc. 1.5% sulfanilamide</td>
<td>54.1</td>
<td>7.44</td>
<td>21.0</td>
<td>100</td>
</tr>
<tr>
<td>150</td>
<td>Infuse: 1.2% sulfanilamide; 10 cc. per minute</td>
<td>55.2</td>
<td>7.44</td>
<td>21.0</td>
<td>100</td>
</tr>
<tr>
<td>165-175</td>
<td>56.2</td>
<td>7.44</td>
<td>20.6</td>
<td>101</td>
<td>30.4</td>
</tr>
<tr>
<td>175-185</td>
<td>55.6</td>
<td>7.44</td>
<td>20.7</td>
<td>101</td>
<td>45.1</td>
</tr>
<tr>
<td>185-205</td>
<td>54.8</td>
<td>7.45</td>
<td>20.4</td>
<td>101</td>
<td>62.0</td>
</tr>
</tbody>
</table>

When sulfanilamide is administered to an animal in acidosis whose renal mechanism for excretion of titratable acid has been loaded by the infusion of phosphate, the capacity of the kidney to eliminate acid is reduced (13, 15). As is evident from experiment 5 in table 3, the administration of sulfanilamide to a normal animal reduces the capacity of the kidney to reabsorb bicarbonate and causes the excretion of bicarbonate and the formation of an alkaline urine. In other experiments in which bicarbonate was administered at rates sufficient to saturate the reabsorptive mechanism, sulfanilamide likewise depressed reabsorptive capacity. However, the extent of the depression in reabsorptive capacity is small in either circumstance, as would be expected if the majority of the bicarbonate were reabsorbed by a non-sulfanilamide-sensitive proximal tubular mechanism. We infer that the extra moiety of bicarbonate excreted after sulfanilamide administration is mainly subject to an anisohydric reabsorptive mechanism rather than to the normal isohydric one.
nilamid e is that which is normally reabsorbed by a process of distal tubular exchange of $\Pi^+$ ions for $Na^+$ ions. Undoubtedly blockage of this mechanism is incomplete at the plasma concentrations of sulfanilamide attained in this experiment (4, 15). It is worthy of note that sulfanilamide did not effect the reabsorption or excretion of chloride, a point of significance to which we shall return later.

Another line of evidence implicates the mechanism of exchange of $H^+$ ion for $Na^+$ ion in the distal tubular reabsorption of bicarbonate. In experiment 6 of table 4 sodium phosphate was infused into an acidotic animal at such a rate as to maintain the plasma concentration of phosphate at a value some 5 times the normal throughout the course of the experiment. In the initial two control periods titratable acid was excreted at an average rate of 0.303 milliequivalent per minute. The plasma bicarbonate was then elevated gradually from its initial low value to a value well within the range of normal by the infusion of sodium bicarbonate. The titratable acidity of the urine diminished and the pH of the urine rose as the quantity of filtered bicarbonate increased. Yet throughout periods 3 to 5 reabsorption of bicarbonate was nearly complete and little appeared in the urine. Only in periods 6 to 8 was excretion of bicarbonate significant. It is evident that the reabsorption of increasing quantities of bicarbonate in periods 3 to 5, unaccompanied by any increase in excretion, in some way diminished the quantity of titratable acid eliminated. We infer that both bicarbonate and phosphate serve as sources of $Na^+$ ions which are exchanged for $H^+$ ions across the tubular epithelium. If little bicarbonate and much phosphate are present in

\[\text{TABLE 4}\]

*Experiment illustrating the depression of the excretion of titratable acid which is produced by the renal reabsorption of progressively increasing quantities of bicarbonate in the dog*

<table>
<thead>
<tr>
<th>TOTAL CONCURRENT TIME</th>
<th>GLOMERULAR FILTRATION RATE</th>
<th>ARTERIAL PLASMA CONCENTRATION</th>
<th>URINE</th>
<th>URINARY EXCRETION RATE</th>
<th>BICARBONATE</th>
<th>REABSORBED</th>
<th>EXCRETED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
<td>Bicarbonate</td>
<td>Phosphate</td>
<td>Flow</td>
<td>pH</td>
<td>Creatinine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mM./l.</td>
<td>mM./l.</td>
<td>cc./min.</td>
<td></td>
<td>mM./min.</td>
</tr>
<tr>
<td>75</td>
<td>Infuse: 0.04 M PO₄; 10 cc. per minute</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100-110</td>
<td>73.2</td>
<td>7.25</td>
<td>12.2</td>
<td>5.48</td>
<td>9.2</td>
<td>4.98</td>
<td>0.188</td>
</tr>
<tr>
<td>110-120</td>
<td>74.9</td>
<td>7.25</td>
<td>12.2</td>
<td>5.51</td>
<td>8.9</td>
<td>4.94</td>
<td>0.200</td>
</tr>
<tr>
<td>121 Infuse: 0.04 M PO₄, 0.22 M NaHCO₃; 10 cc. per minute</td>
<td>88.3</td>
<td>7.48</td>
<td>22.9</td>
<td>5.44</td>
<td>9.27</td>
<td>6.03</td>
<td>0.215</td>
</tr>
<tr>
<td>130-140</td>
<td>85.2</td>
<td>7.38</td>
<td>18.2</td>
<td>5.29</td>
<td>7.7</td>
<td>5.78</td>
<td>0.225</td>
</tr>
<tr>
<td>140-150</td>
<td>81.8</td>
<td>7.44</td>
<td>21.0</td>
<td>5.29</td>
<td>7.43</td>
<td>6.27</td>
<td>0.215</td>
</tr>
<tr>
<td>150-161</td>
<td>88.3</td>
<td>7.46</td>
<td>22.9</td>
<td>5.44</td>
<td>9.27</td>
<td>6.03</td>
<td>0.248</td>
</tr>
<tr>
<td>161-170</td>
<td>86.6</td>
<td>7.46</td>
<td>24.3</td>
<td>5.18</td>
<td>7.8</td>
<td>6.82</td>
<td>0.238</td>
</tr>
<tr>
<td>170-180</td>
<td>80.6</td>
<td>7.46</td>
<td>23.9</td>
<td>5.18</td>
<td>7.8</td>
<td>6.82</td>
<td>0.238</td>
</tr>
<tr>
<td>180-190</td>
<td>76.2</td>
<td>7.49</td>
<td>25.9</td>
<td>4.96</td>
<td>5.9</td>
<td>6.99</td>
<td>0.203</td>
</tr>
</tbody>
</table>

The titratable acid figures are calculated from the pH of the urine and the excretion rate of creatinine and phosphate. Calculated and experimentally determined values agree within ±5 per cent.
the tubular lumen at the site of the urinary acidification, large quantities of 
secondary phosphate are transformed into the primary form and the titratable 
acidity of the urine is high. Such conditions are to be expected in acidosis. On 
the other hand, if much bicarbonate is present in the tubular urine, a part is 
transformed into carbonic acid, relatively less of the phosphate is changed, and the 
titratable acidity of the urine is correspondingly reduced. Such conditions are to 
be expected in alkalosis.

**DISCUSSION.** Three characteristics of the renal tubular mechanism for the 
reabsorption of bicarbonate admirably fit it for its function of stabilizing the 
alkali reserve of the plasma. 1. The mechanism is remarkably efficient in reab-
sorbing all but a trace of bicarbonate from the tubular urine when the plasma 
concentration is below normal. Thus none of the depleted body store is wasted. 
2. The mechanism is capable of reabsorbing on an average 2.5 millimols of bicar-
bonate from each 100 cc. of glomerular filtrate. When more than this quantity 
is delivered into the glomerular filtrate as a consequence of an increase in plasma 
concentration, the excess is excreted in the urine. Accordingly the plasma bicar-
bonate concentration tends to stabilize at 2.5 millimols per 100 cc., or as usually 
expressed, at 25 millimols per liter, when adequate supplies of base are available 
to the body. 3. The quantity of bicarbonate reabsorbed per unit of time varies 
directly and nearly proportionally with changes in the rate of glomerular filtra-
tion. Consequently the renal bicarbonate threshold is relatively stable and 
nearly independent of the glomerular filtration rate.

This latter characteristic sets the renal mechanism for reabsorption of bicar-
bonate apart from those for reabsorption of glucose (18), vitamin C (19), and 
phosphate (14). The reabsorptive capacities of these latter mechanisms are 
fixed and independent of both glomerular filtration rate and plasma concentra-
tion (above the renal threshold). It has been generally assumed that this type of 
reabsorptive limitation derives from the presence within the renal tubular cells of 
a fixed quantity of some cellular component with which the reabsorbed material 
combines in the process of tubular transfer (17). The rate of breakdown of this 
tubular complex presumably limits the overall rate of reabsorption. Such a 
simple explanation obviously will not account for the reabsorption of bicarbonate. 
Although the quantity of bicarbonate reabsorbed per unit of time is independent 
of plasma concentration above the renal threshold, it varies directly with the 
filtration rate.

In attempting to visualize a means by which reabsorptive capacity could be 
correlated with filtration rate we have arrived at the following modification of 
Cushny's concept of the tubular reabsorption of fluid of constant composition 
(3). It is generally assumed that four-fifths of the water filtered through the 
glomeruli is reabsorbed in its passage through the proximal tubules by an obliga-
tory process (20). If this were true at all rates of glomerular filtration, and if the 
concentration of bicarbonate in this reabsorbate were limited to a value of not 
more than 2.5 millimols per 100 cc., the quantity of bicarbonate reabsorbed in 
the proximal tubule per unit of time would vary in proportion to the glomerular 
filtration rate. How the concentration of bicarbonate in the proximal reab-
sorbate is limited to 2.5 millimols per 100 cc. is beyond our present comprehension of the problem. However, as a consequence of the operation of such a mechanism up to 2.0 millimols of bicarbonate could be reabsorbed in the proximal tubule from each 100 cc. of glomerular filtrate, i.e., 80 cc. of fluid containing 2.5 millimols per 100 cc. At normal plasma concentrations, roughly 0.5 millimol of bicarbonate would be delivered into the distal tubule for each 100 cc. of original filtrate. If we assume that the distal tubule can reabsorb quantities of this order of magnitude, then the urine will be essentially free of bicarbonate. When more than 0.5 millimol reaches the distal tubule, the excess is excreted. Since proximal reabsorption is quantitatively much greater than distal reabsorption, total bicarbonate reabsorption would vary in rough proportion to glomerular filtration rate no matter what the characteristics of the distal mechanism might be.

There is reason for believing that the distal reabsorptive mechanism differs from the proximal mechanism in at least two ways. 1. Distal reabsorption of bicarbonate is nearly, although not completely, independent of water reabsorption, whereas proximal reabsorption obviously is proportional to water reabsorption. The independence of distal reabsorption of water and of bicarbonate derives from the fact that in man urine flow can be varied over a wide range with only small changes in urine pH and hence only small changes in bicarbonate excretion (1). Since variations in urine flow are effected largely through inverse variations in the quantity of water reabsorbed in the distal tubule (20), it is evident that water and bicarbonate reabsorption are relatively independent. 2. Distal reabsorption of bicarbonate is an anisohydric process, whereas proximal reabsorption is probably an isohydric process. Evidence presented in this paper supports the view that bicarbonate reabsorption is effected indirectly in the distal tubule by the exchange of H+ ions for Na+ ions, thereby converting bicarbonate in the tubular urine to carbonic acid. This carbonic acid on dehydration to carbon dioxide diffuses across the tubular epithelium into the renal venous blood. Failure to establish equilibrium across the tubular epithelium would account for the fact that the pCO₂ of urine is often higher than that of blood, especially when the distal mechanism is loaded and bicarbonate appears in the urine.

We have no direct evidence as to the nature of the proximal reabsorptive mechanism in the dog. Reabsorption in this segment of the amphibian kidney occurs isohydrically (11), and we presume the same to be true of the mammalian kidney within limits. However, experiments 3 and 4 indicate that there is a mutual interference in the reabsorption of bicarbonate and chloride when either anion is present in the tubular urine in large excess, and experiment 5 suggests that the site of this interaction is in the proximal, not the distal, tubule. Sulfanilamide, which presumably depresses the distal reabsorption of bicarbonate, has no effect on chloride reabsorption. From this we conclude that the distal chloride and distal bicarbonate reabsorptive mechanisms are independent, and that interaction must occur in the proximal tubule.

**SUMMARY**

The renal tubular reabsorption of bicarbonate in the normal dog has been assessed at plasma concentrations ranging from 10 to 70 millimols per liter.
1. Under the conditions of our experiments the renal threshold for gross excretion of bicarbonate is approximately 25 millimols per liter of plasma. Below the renal threshold essentially all of the filtered bicarbonate is reabsorbed. Above the renal threshold the rate of excretion of bicarbonate is a linear function of the plasma concentration.

2. On an average the renal tubules reabsorb 2.5 millimols of bicarbonate from each 100 cc. of filtrate when the plasma concentration is above the threshold. The excess filtered, over and above this limited quantity reabsorbed, is excreted in the urine.

3. Although the capacity of the tubules to reabsorb bicarbonate is independent of plasma concentration above the threshold, it is not fixed in the ordinary sense of Tm. Functional increases in filtration rate are accompanied by essentially equivalent increases in tubular reabsorptive capacity. Thus the quantity reabsorbed per 100 cc. of filtrate remains the same, and the renal threshold is independent of filtration rate.

4. The renal thresholds for chloride and bicarbonate are interrelated in such a fashion as to maintain constant the sum of the plasma concentrations of these two anions. Thus an increase in plasma chloride concentration reduces the renal threshold for bicarbonate, and conversely an increase in plasma bicarbonate concentration reduces the renal threshold for chloride.

5. The partial pressure of carbon dioxide in acid urines approximates that of the arterial plasma. In alkaline urines it exceeds that of the plasma by a considerable margin, attaining a maximum observed value of 109 mm. Hg. Under the conditions of our experiments acid urines contain a minimum of 0.08 millimol of bicarbonate per liter, whereas alkaline urines contain a maximum of 197 millimols per liter. Urine pH accordingly varies within limits of 4.96 and 7.96.

6. In acidosis the rate of excretion of titratable acid varies inversely with the quantity of bicarbonate reabsorbed by the renal tubules, even though the rate of excretion of bicarbonate is low and remains essentially unchanged. The administration of large amounts of sulfanilamide not only reduces the rate of excretion of titratable acid but reduces the capacity of the renal tubules to reabsorb bicarbonate. We interpret these two observations as indicating that bicarbonate is reabsorbed in small part by that distal tubular mechanism which is responsible for the elimination of titratable acid.

7. We infer from our data that a much larger moiety of bicarbonate is reabsorbed by an independent proximal tubular mechanism, the characteristics of which largely determine the overall nature of bicarbonate reabsorption as outlined in sections 1 to 4 above.

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