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## THE RELATION OF URINARY CO<sub>2</sub> TENSION TO BICARBONATE EXCRETION \*

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The mechanism whereby high CO<sub>2</sub> tensions are formed in the urine has been the subject of considerable debate. Pitts and Alexander (1) proposed that the CO<sub>2</sub> tension in alkaline urines is increased as a result of the following sequence: 1) increased delivery of NaHCO<sub>3</sub> to the distal tubule; 2) increased formation of H<sub>2</sub>CO<sub>3</sub> as a result of a Na<sup>+</sup>-H<sup>+</sup> exchange in this segment; 3) delayed dehydration of the H<sub>2</sub>CO<sub>3</sub> thus formed. More recently, Kennedy, Eden and Berliner (2) have emphasized the role of nonbicarbonate buffer as the factor responsible for delayed dehydration of H<sub>2</sub>CO<sub>3</sub>. It is apparent, therefore, that variations in the excretion of buffer as well as bicarbonate might influence the urinary CO<sub>2</sub> tension.

The high CO<sub>2</sub> tensions that follow the administration of Diamox® are particularly difficult to explain within this conceptual framework, because Diamox® undeniably suppresses overall renal H<sup>+</sup> secretion. Evidence has recently been presented (3) to support the thesis that the high CO<sub>2</sub> tension in urine during a bicarbonate diuresis induced by Diamox® is the consequence, not of the enhanced bicarbonate excretion, but of the buffering action of the inhibitor.

The present studies were designed to examine in man the relationship of buffer and bicarbonate excretion to urine CO<sub>2</sub> tension by observing the pattern of various types of bicarbonate diuresis, including that produced by Diamox®, with and without the simultaneous infusion of phosphate buffer.

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### MATERIALS AND METHODS

A total of 27 experiments were performed on normal young adults (medical students, house officers and student nurses) after an overnight fast.

Maximum water diuresis was maintained by the rapid intravenous infusion of either 5 per cent dextrose or 5 per cent fructose in water. The subjects remained recumbent throughout the experiments except for those individuals from whom voided urine specimens were obtained. Urine collection periods of 15 to 20 minutes were employed; the samples were collected under mineral oil by direct voiding in some experiments, by indwelling catheter in most. At the mid-point of each urine collection period blood was drawn into heparinized, oiled syringes from an indwelling needle in either a brachial or femoral artery.

Blood and urine pH were anaerobically determined immediately with a Cambridge pH meter with the electrode assembly housed in an incubator at a constant temperature of 37° C. Plasma and urine CO<sub>2</sub> content were determined by the method of Van Slyke and Neill (4). Bicarbonate concentration and pCO<sub>2</sub> were calculated from the Henderson-Hasselbalch equation. The pK<sub>1</sub> of H<sub>2</sub>CO<sub>3</sub> in urine was estimated for each sample according to the formula:  $pK_1 = 6.33 - 0.5 \sqrt{B}$ , where B represents the total cation concentration of the sample in question (5). It was assumed that the sum [Na<sup>+</sup>]+[K<sup>+</sup>] was equal to at least 95 per cent of [B<sup>+</sup>]. It was found that the arbitrary selection of a pK<sub>1</sub> of 6.10 introduced a variable error of considerable magnitude when calculating pCO<sub>2</sub> in urines of varying pH and bicarbonate concentration. However, using the pK<sub>1</sub> estimated from  $6.33 - 0.5 \sqrt{B}$ , close agreement was obtained between calculated pCO<sub>2</sub> and tonometrically determined pCO<sub>2</sub>. The conventional proportionality constant of 0.0309 was used to convert [H<sub>2</sub>CO<sub>3</sub>] to pCO<sub>2</sub>. For plasma, a pK<sub>1</sub> of 6.10 and a proportionality constant of 0.0301 were used.

Inorganic phosphate in urine was determined by the method of Fiske and Subbarow (6). Titration curves for urines obtained during several of the experiments were determined as follows:

Ten ml. urine was acidified to < pH 2.0 by the addition of 0.1 HCl. Following vigorous stirring for 15 minutes (to dispel generated CO<sub>2</sub>), the samples were titrated to pH 6.0 to 6.2 with 0.1 N NaOH. Further titration to pH > 8.0 was performed with 0.01 N NaOH which was

TABLE I  
The relation of U-P pCO<sub>2</sub> to bicarbonate excretion during maximal water diuresis

| Subject,<br>experiment                            | Time        | Plasma                                               |                               |                  | Urine            |                         |      |                               |                                |              |                |                 |
|---------------------------------------------------|-------------|------------------------------------------------------|-------------------------------|------------------|------------------|-------------------------|------|-------------------------------|--------------------------------|--------------|----------------|-----------------|
|                                                   |             | pH                                                   | HCO <sub>3</sub> <sup>-</sup> | pCO <sub>2</sub> | pCO <sub>2</sub> | U—P<br>pCO <sub>2</sub> | pH   | HCO <sub>3</sub> <sup>-</sup> | PO <sub>4</sub> <sup>'''</sup> | Flow         |                |                 |
|                                                   | <i>min.</i> |                                                      | <i>mEq./L.</i>                | <i>mm. Hg</i>    | <i>mm. Hg</i>    | <i>mm. Hg</i>           |      | <i>mEq./L.</i>                | <i>μEq./min.</i>               | <i>mM/L.</i> | <i>μM/min.</i> | <i>ml./min.</i> |
| C. G.<br>Diamox®                                  | 0—15        | 7.35                                                 | 23.9                          | 44               | 57               | 13                      | 6.22 | 1.8                           | 53                             | 0.5          | 15             | 29.3            |
|                                                   | 16—35*      | 7.34                                                 | 23.1                          | 44               | 47               | 3                       | 6.36 | 2.1                           | 95                             | 0.2          | 9              | 45.0            |
|                                                   | 36—55       | 7.35                                                 | 23.1                          | 43               | 64               | 21                      | 7.11 | 16.1                          | 383                            | 0.2          | 5              | 23.8            |
|                                                   | 56—75       | 7.34                                                 | 23.1                          | 44               | 66               | 22                      | 7.17 | 19.2                          | 662                            | 0.6          | 21             | 34.5            |
|                                                   | 76—95       | 7.36                                                 | 22.9                          | 42               | 67               | 25                      | 7.08 | 15.6                          | 468                            | 0.5          | 15             | 30.0            |
|                                                   | 96—115      | 7.33                                                 | 22.8                          | 44               | 77               | 33                      | 6.98 | 14.3                          | 422                            | 0.7          | 21             | 29.5            |
| J. H.<br>Metabolic<br>alkalosis†                  | —40         | Begin infusion 7.5% NaHCO <sub>3</sub> at 6 ml./min. |                               |                  |                  |                         |      |                               |                                |              |                |                 |
|                                                   | 0—20        | 7.41                                                 | 27.4                          | 45               | 62               | 17                      | 7.23 | 20.6                          | 268                            | 1.9          | 25             | 13.0            |
|                                                   | 21—40       | 7.42                                                 | 30.8                          | 49               | 78               | 29                      | 7.38 | 36.3                          | 490                            | 1.5          | 20             | 13.5            |
|                                                   | 41—60       | 7.45                                                 | 31.5                          | 47               | 89               | 42                      | 7.45 | 49.0                          | 564                            | 1.7          | 20             | 11.5            |
|                                                   | 61—80       | 7.52                                                 | 32.8                          | 42               | 88               | 46                      | 7.49 | 53.1                          | 770                            | 1.6          | 23             | 14.5            |
| J. W.<br>Respiratory<br>alkalosis                 | 81—100      | 7.45                                                 | 35.2                          | 52               | 90               | 38                      | 7.55 | 62.6                          | 864                            | 1.5          | 21             | 13.8            |
|                                                   | 0—20        | 7.39                                                 | 23.4                          | 39               | 44               | 5                       | 5.81 | 0.6                           | 14                             |              |                | 21.5            |
|                                                   | 21—40‡      | 7.38                                                 | 24.0                          | 40               | 41               | 1                       | 6.01 | 0.9                           | 24                             |              |                | 25.0            |
|                                                   | 41—60       | 7.50                                                 | 19.3                          | 26               | 28               | 2                       | 6.35 | 1.3                           | 38                             |              |                | 29.0            |
|                                                   | 61—80       | 7.52                                                 | 19.0                          | 25               | 24               | —1                      | 6.60 | 2.0                           | 52                             |              |                | 26.0            |
|                                                   | 81—100‡     | 7.49                                                 | 17.7                          | 24               | 22               | —2                      | 6.58 | 1.8                           | 40                             |              |                | 22.5            |
| C. D.<br>Metabolic<br>alkalosis§<br>+<br>Diamox®  | 101—120     | 7.39                                                 | 22.0                          | 37               | 36               | —1                      | 6.56 | 2.5                           | 72                             |              |                | 29.0            |
|                                                   | 121—140     | 7.37                                                 | 23.6                          | 39               | 31               | —8                      | 6.50 | 2.0                           | 44                             |              |                | 22.0            |
|                                                   | 0—20        | 7.41                                                 | 24.9                          | 40               | 56               | 16                      | 6.28 | 2.1                           | 66                             |              |                | 31.5            |
|                                                   | 21—40       | 200 ml. 7.5% NaHCO <sub>3</sub> I.V.                 |                               |                  |                  |                         |      |                               |                                |              |                |                 |
|                                                   | 41—60*      | 7.47                                                 | 36.3                          | 51               | 97               | 46                      | 7.49 | 65.9                          | 1,865                          |              |                | 28.3            |
|                                                   | 61—80       | 7.50                                                 | 36.4                          | 48               | 68               | 20                      | 7.65 | 65.9                          | 2,069                          |              |                | 31.4            |
| J. W.<br>Respiratory<br>alkalosis<br>+<br>Diamox® | 81—100      | 7.49                                                 | 32.5                          | 46               | 66               | 20                      | 7.63 | 61.8                          | 1,452                          |              |                | 23.5            |
|                                                   | 101—120     | 7.50                                                 | 34.7                          | 46               | 70               | 24                      | 7.62 | 64.1                          | 1,699                          |              |                | 26.5            |
|                                                   | 0—24        | 7.40                                                 | 24.7                          | 41               | 39               | —2                      | 6.01 | 0.9                           | 19                             |              |                | 20.4            |
|                                                   | 25—53*      | 7.41                                                 | 25.6                          | 42               | 45               | 3                       | 5.83 | 0.7                           | 13                             |              |                | 18.3            |
|                                                   | 54—76‡      | 7.37                                                 | 23.6                          | 42               | 62               | 20                      | 7.17 | 18.2                          | 546                            |              |                | 30.0            |
|                                                   | 77—98       | 7.51                                                 | 17.1                          | 22               | 48               | 26                      | 7.34 | 20.8                          | 449                            |              |                | 21.6            |
| N. K.<br>Respiratory<br>acidosis<br>+<br>Diamox®  | 99—118      | 7.48                                                 | 18.2                          | 25               | 47               | 22                      | 7.39 | 22.6                          | 593                            |              |                | 26.2            |
|                                                   | 119—139‡    | 7.54                                                 | 15.5                          | 19               | 45               | 26                      | 7.34 | 18.4                          | 648                            |              |                | 35.2            |
|                                                   | 140—161     | 7.38                                                 | 19.8                          | 34               | 47               | 13                      | 7.16 | 13.4                          | 341                            |              |                | 25.4            |
|                                                   | 162—181     | 7.35                                                 | 20.4                          | 38               | 52               | 14                      | 6.98 | 9.8                           | 290                            |              |                | 29.5            |
|                                                   | 0—15        | 7.38                                                 | 23.5                          | 41               | 52               | 11                      | 6.33 | 2.2                           | 60                             | 0.1          | 3              | 27.3            |
|                                                   | 16—30*      | 7.39                                                 | 23.6                          | 40               | 59               | 19                      | 6.27 | 2.1                           | 47                             |              |                | 22.3            |
| J. H.<br>Respiratory<br>acidosis                  | 31—45       | 7.39                                                 | 23.7                          | 40               | 65               | 25                      | 7.16 | 18.3                          | 512                            | 0.05         | 1              | 28.0            |
|                                                   | 46—60       | 7.27                                                 | 24.8                          | 56               | 83               | 27                      | 7.20 | 25.8                          | 697                            | 0.3          | 8              | 27.0            |
|                                                   | 61—75       | 7.23                                                 | 24.0                          | 59               | 94               | 35                      | 7.07 | 21.5                          | 688                            | 0.4          | 13             | 32.0            |
|                                                   | 76—90       | 7.34                                                 | 21.5                          | 41               | 76               | 35                      | 7.16 | 21.4                          | 456                            | 0.7          | 15             | 21.3            |
|                                                   | 91—105      | 7.41                                                 | 22.6                          | 37               | 94               | 57                      | 7.26 | 33.5                          | 425                            | 1.0          | 13             | 12.7            |
|                                                   | 0—20        | 7.40                                                 | 23.2                          | 39               | 48               | 9                       | 6.32 | 2.0                           | 38                             |              |                | 19.0            |
| Respiratory<br>acidosis                           | 21—40       | 7.32                                                 | 25.1                          | 50               | 60               | 10                      | 6.35 | 2.6                           | 49                             |              |                | 19.0            |
|                                                   | 41—55       | 7.27                                                 | 25.1                          | 56               | 67               | 11                      | 6.23 | 2.2                           | 47                             |              |                | 21.3            |
|                                                   | 56—70       | 7.23                                                 | 25.7                          | 63               | 68               | 5                       | 6.19 | 2.1                           | 43                             |              |                | 20.7            |
|                                                   | 71—85       | 7.35                                                 | 24.2                          | 45               | 66               | 21                      | 6.44 | 3.6                           | 54                             |              |                | 15.3            |

\* Injection of 250 mg. Diamox® intravenously.

† The infusion of 7.5 per cent NaHCO<sub>3</sub> was given at the rate of 6 ml. per minute for 40 minutes before urines were collected and continued throughout the experiment at this rate.

‡ Period of hyperventilation.

§ The infusion of 7.5 per cent NaHCO<sub>3</sub> was given at the rate of 10 ml. per minute for 19 minutes, after which the rate was slowed to 5 ml. per minute.

|| Period of breathing 6 per cent CO<sub>2</sub>.

added in small increments with pH determination following each addition.

Eight types of experiments, as charted below, were performed to examine the relationship between bicarbonate excretion and the urine minus plasma (U-P) pCO<sub>2</sub> gradient under conditions of varying plasma acid-base composition and varying bicarbonate and buffer excretion.

#### Type

Respiratory alkalosis  
Respiratory alkalosis + Diamox®  
Respiratory acidosis  
Respiratory acidosis + Diamox®  
Metabolic alkalosis  
Metabolic alkalosis + Diamox®  
Diamox® only  
Diamox® + phosphate infusion  
Metabolic alkalosis + phosphate infusion

Respiratory alkalosis was induced by voluntary hyperventilation, assisted by a Haliburton IPPB machine, respiratory acidosis by breathing 6 to 6.5 per cent CO<sub>2</sub> in oxygen. Metabolic alkalosis was produced by the intravenous infusion of hypertonic (7.5 per cent) NaHCO<sub>3</sub> at rates of 2 to 6 ml. per minute. The phosphate solutions used were made from Na<sub>2</sub>HPO<sub>4</sub>-NaH<sub>2</sub>PO<sub>4</sub> (pH 7.4), diluted to isotonicity and delivered at rates of 4 to 8 ml. per minute. Diamox® was given intravenously as the sodium salt in a single injection of 250 mg.<sup>1</sup>

#### RESULTS

Representative experiments are charted in Table I. Urine pCO<sub>2</sub> was increased following the in-

<sup>1</sup> The authors wish to thank Lederle Laboratories Division, American Cyanamid Company, for generously supplying the Diamox® used in these experiments.

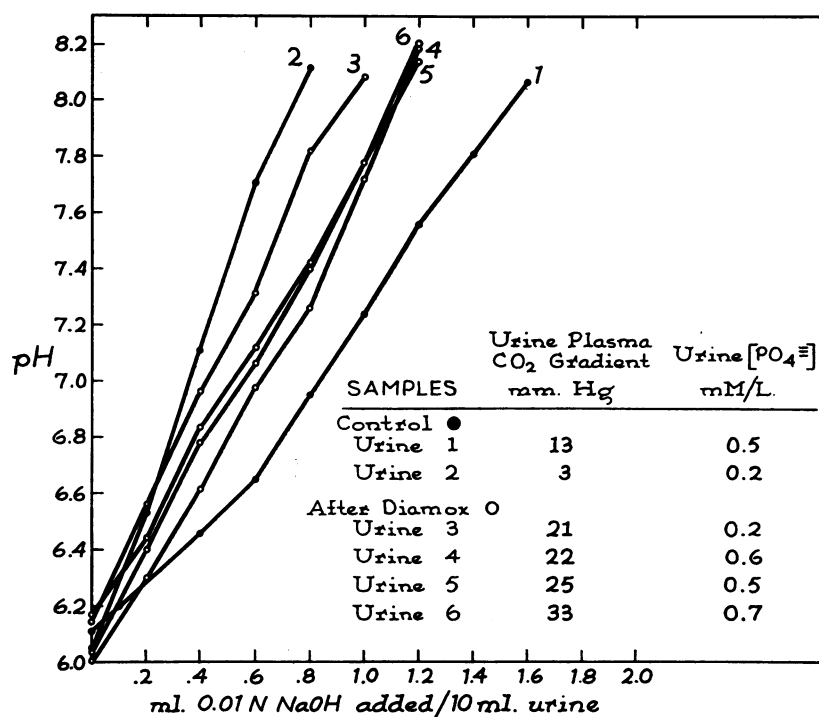


FIG. 1. TITRATION CURVES OF URINES OBTAINED BEFORE AND AFTER DIAMOX® ADMINISTRATION DURING MAXIMAL WATER DIURESIS

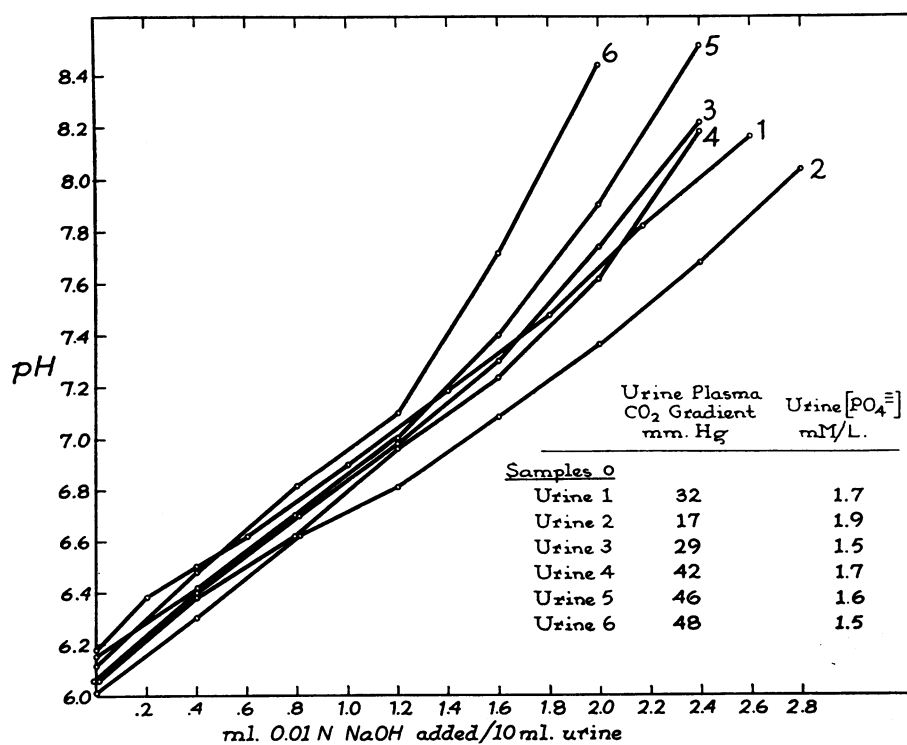


FIG. 2. TITRATION CURVES OF URINES OBTAINED DURING BICARBONATE DIURESIS AND MAXIMAL WATER DIURESIS

TABLE II  
Effects of massive buffer excretion on U-P pCO<sub>2</sub> during bicarbonate diuresis

| Subject,<br>experiment                                              | Time        | Plasma                                        |                               |                  | Urine            |                         |      |                               |                              |              |                |                 |
|---------------------------------------------------------------------|-------------|-----------------------------------------------|-------------------------------|------------------|------------------|-------------------------|------|-------------------------------|------------------------------|--------------|----------------|-----------------|
|                                                                     |             | pH                                            | HCO <sub>3</sub> <sup>-</sup> | pCO <sub>2</sub> | pCO <sub>2</sub> | U-P<br>pCO <sub>2</sub> | pH   | HCO <sub>3</sub> <sup>-</sup> | PO <sub>4</sub> <sup>■</sup> | Flow         |                |                 |
|                                                                     | <i>min.</i> |                                               | <i>mEq./L.</i>                | <i>mm. Hg</i>    | <i>mm. Hg</i>    | <i>mm. Hg</i>           |      | <i>mEq./L.</i>                | <i>μEq./min.</i>             | <i>mM/L.</i> | <i>μM/min.</i> | <i>ml./min.</i> |
| L. J.                                                               | 0- 20       | 7.39                                          | 21.1                          | 36               | 42               | 6                       | 6.22 | 1.3                           | 22                           | 0.6          | 10             | 17.0            |
| Diamox <sup>®</sup><br>only                                         | 21- 40*     | 7.37                                          | 21.1                          | 38               | 50               | 12                      | 6.08 | 1.2                           | 17                           | 0.6          | 8              | 14.0            |
|                                                                     | 41- 60      | 7.36                                          | 20.9                          | 38               | 56               | 18                      | 6.98 | 10.4                          | 222                          | 0.8          | 17             | 21.3            |
|                                                                     | 61- 80      | 7.36                                          | 19.1                          | 35               | 63               | 28                      | 7.27 | 22.9                          | 573                          | 1.0          | 25             | 25.0            |
|                                                                     | 81-100      | 7.38                                          | 19.2                          | 33               | 64               | 21                      | 7.27 | 23.1                          | 462                          | 1.0          | 20             | 20.0            |
|                                                                     | 101-120     |                                               | 20.7                          | 35               | 63               | 28                      | 7.24 | 21.5                          | 441                          | 1.0          | 20             | 20.5            |
| L. J.                                                               | - 40        | Start isotonic buffer phosphate at 8 ml./min. |                               |                  |                  |                         |      |                               |                              |              |                |                 |
| Diamox <sup>®</sup><br>+<br>massive<br>PO <sub>4</sub><br>infusion† | 0- 20       | 7.36                                          | 19.7                          | 36               | 37               | 1                       | 5.88 | 0.6                           | 7                            |              |                | 11.0            |
|                                                                     | 21- 40*     | 7.34                                          | 20.0                          | 38               | 36               | -2                      | 5.75 | 0.4                           | 5                            | 17.6         | 229            | 13.0            |
|                                                                     | 41- 60      | 7.35                                          | 20.1                          | 38               | 96               | 58                      | 6.84 | 12.9                          | 271                          | 16.6         | 349            | 21.0            |
|                                                                     | 61- 80      | 7.34                                          | 20.0                          | 38               | 74               | 36                      | 7.06 | 16.7                          | 276                          | 19.5         | 322            | 16.5            |
|                                                                     | 81-100      | 7.36                                          | 20.0                          | 37               | 82               | 45                      | 7.00 | 16.1                          | 282                          | 20.7         | 362            | 17.5            |
|                                                                     | 101-120     | 7.34                                          | 20.1                          | 39               | 77               | 38                      | 7.04 | 16.3                          | 274                          | 18.1         | 304            | 16.8            |

\* Injection of 250 mg. Diamox® intravenously.

† Isotonic sodium phosphate (pH 7.4) was infused at the rate of 8 ml. per minute for 40 minutes before the start of urine collections and was continued at this rate throughout the experiment.

jection of Diamox® (C.G.), during NaHCO<sub>3</sub> infusion (J.H.), during NaHCO<sub>3</sub> infusion and Diamox® administration (C.D.), during respiratory acidosis with Diamox® (N.K.) and during respiratory acidosis alone (J.H.). It was decreased during respiratory alkalosis alone (J.W.) and was not appreciably increased during respiratory alkalosis plus Diamox® (J.W.).

These changes in the absolute value of urine pCO<sub>2</sub> cannot automatically be interpreted to indicate increased generation of CO<sub>2</sub> from H<sub>2</sub>CO<sub>3</sub> formed within the tubular lumen. Since CO<sub>2</sub> diffuses freely across cell membranes, alterations in plasma pCO<sub>2</sub> will change the pCO<sub>2</sub> in urine without any associated change in intraluminal H<sub>2</sub>CO<sub>3</sub> production. To correct for this, pCO<sub>2</sub> has been expressed as the difference between urine and plasma CO<sub>2</sub> tension (7). The ratio U/P pCO<sub>2</sub> (2) has not been used because the same U-P gradient might result in different ratios. In the last period of Experiment C.G., U-P was 33 mm. and U/P was 1.75. In the last hyperventilation period of J.W., U-P was 26 mm., but a higher U/P ratio of 2.37 was observed.

It is clear from Table I that appreciable bicarbonate diuresis, no matter how induced, was invariably associated with a definite increase in U-P gradient. The urine concentration of phosphate was exceedingly low throughout the studies and did not increase appreciably during the experiments in which phosphate was not infused.

To determine whether enhanced excretion of buffers other than phosphate might account for

the high U-P gradients during NaHCO<sub>3</sub> diuresis, titration curves were determined from urines obtained following Diamox® administration (Figure 1) and NaHCO<sub>3</sub> infusion (Figure 2). Despite the fact that a very dilute solution of NaOH was used to titrate the urines, no increases in buffer concentration between control and experimental samples could be detected. Nevertheless, marked increases in U-P pCO<sub>2</sub> occurred. Similar curves were obtained in all experiments where bicarbonate diuresis was produced without phosphate loads.

That great increases in buffer excretion do, in fact, enhance U-P is seen in the representative experiments listed in Table II, where phosphate excretion was increased 20-fold by a phosphate infusion administered during a Diamox® induced diuresis. A similar effect of phosphate was found in other studies where NaHCO<sub>3</sub> and phosphate were infused.

Figure 3 illustrates the relation between bicarbonate excretion and U-P pCO<sub>2</sub> for all studies. Under the conditions of these experiments, where maximal water diuresis was maintained, a mean gradient of about 30 mm. Hg was obtained at bicarbonate excretion of approximately 300 μEq. per minute, irrespective of the manner in which bicarbonate diuresis was produced. Increasing bicarbonate excretion beyond this point did not increase U-P.

#### DISCUSSION

An increase in the CO<sub>2</sub> tension of the urine may result from several different processes. First, ele-

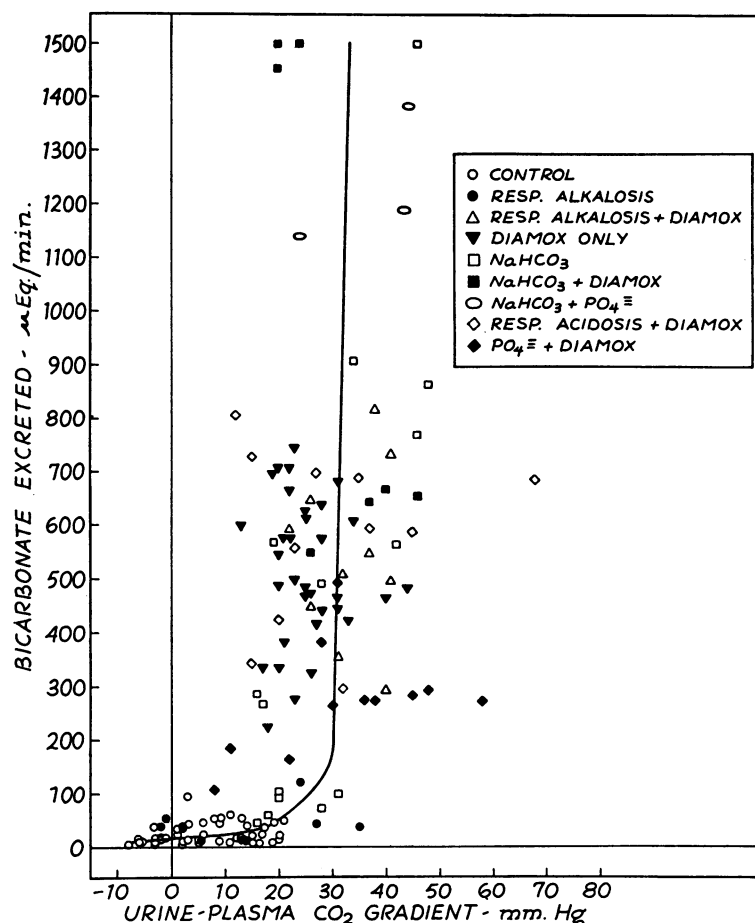


FIG. 3. RELATIONSHIP BETWEEN  $\text{HCO}_3^-$  EXCRETION AND URINE MINUS PLASMA  $\text{CO}_2$  GRADIENT DURING MAXIMAL WATER DIURESIS

variations of plasma  $\text{pCO}_2$  during respiratory acidosis would increase urinary  $\text{CO}_2$  tension because of the rapid diffusion of  $\text{CO}_2$  between renal blood and tubular urine. Second, the mixture in the bladder of urines of alkaline and acid pH, such as occurs during prolonged urine collection periods when plasma acid-base composition is changing, would readily generate high  $\text{CO}_2$  tensions. Third,  $\text{H}_2\text{CO}_3$  might be generated in the collecting ducts as a result of the admixture of acid and alkaline urines originating in heterogeneous nephrons (7). Finally, the secretion of  $\text{H}^+$  by distal tubular cells into luminal fluid containing  $\text{HCO}_3^-$  would also result in the formation of  $\text{H}_2\text{CO}_3$  (1). It should be emphasized that, in order for these last two processes to elevate the U-P gradient, it is necessary that the dehydration of  $\text{H}_2\text{CO}_3$  be delayed. The obliteration of U-P gradients in alkaline

urines following the injection of carbonic anhydrase in amounts sufficient to cause its excretion into the urine (8) strongly supports the central role attributed to delayed dehydration, although it does not establish which of the last two processes is responsible for the formation of  $\text{H}_2\text{CO}_3$ .

The effects of changes in plasma  $\text{CO}_2$  tension are obviated by employing the urine minus plasma (U-P) gradient of  $\text{pCO}_2$  rather than the absolute value of  $\text{pCO}_2$  as a basis for comparison. The elevation of plasma  $\text{CO}_2$  tension during metabolic alkalosis and respiratory acidosis, although raising the  $\text{CO}_2$  tension of the urine, would have no effect on the gradient. In the present studies, the short urine collection periods, maximal water diuresis, and constant experimental circumstances all combine to minimize the effects of bladder mixing. The increased U-P gradient observed in all ex-

periments where noteworthy bicarbonate diuresis occurred is, therefore, attributable to events within the kidney.

Kennedy, Orloff and Berliner (7) have proposed that acid and alkaline urines delivered from heterogeneous nephrons are mixed in the collecting duct system, thereby forming H<sub>2</sub>CO<sub>3</sub> and hence resulting in a high U-P gradient. In addition to the admixture of acid and alkaline urines, the mixing hypothesis requires, as Kennedy and co-workers point out, that the acid urine involved contain some buffer in order to generate increased urinary CO<sub>2</sub> tension. Inasmuch as the urines obtained in the present studies contained so little buffer, it seems unlikely, although not impossible, that admixture of heterogeneous urines could account for the observed gradients.

Under the conditions of the studies, the high U-P gradients are most reasonably attributed to accelerated delivery of NaHCO<sub>3</sub> to the distal tubule, where the reabsorption of Na<sup>+</sup> in exchange for H<sup>+</sup> generated large amounts of H<sub>2</sub>CO<sub>3</sub>. The dehydration of H<sub>2</sub>CO<sub>3</sub> thus formed was delayed, owing to the absence of carbonic anhydrase from tubular urine. Complete dehydration of H<sub>2</sub>CO<sub>3</sub>, therefore, occurs lower in the urinary tract where unfavorable volume-surface relationships, or less permeable epithelium, serves to perpetuate the gradient.

While accepting the thesis that delayed dehydration of H<sub>2</sub>CO<sub>3</sub> is responsible for the high U-P gradients in alkaline urines, Kennedy, Eden and Berliner (2) have presented evidence from *in vitro* studies to show that the dehydration of H<sub>2</sub>CO<sub>3</sub> is immeasurably rapid in the absence of nonbicarbonate buffer. They therefore propose that in order for dehydration of H<sub>2</sub>CO<sub>3</sub> to be delayed, buffer must be present in the tubular urine. It follows from this that in alkaline urines, the U-P gradient might rise as a result of increased buffer excretion independently of changes in the magnitude of bicarbonate diuresis.

In the present study increased U-P gradients following NaHCO<sub>3</sub> infusions were not accompanied by changes in buffer excretion. Moreover, owing to the fasting state and the maximal water diuresis, the concentration of urinary buffer was exceedingly low. It appears, therefore, that very small amounts of buffer will delay the dehydration of H<sub>2</sub>CO<sub>3</sub> sufficiently to generate high U-P gradi-

ents. The fact that massive phosphate excretion resulted in a still further increment in U-P gradient suggests that the excretion of buffer, although influencing urine CO<sub>2</sub> tension to some extent, has only a minor effect in the range of buffer excretion ordinarily encountered.

It is especially noteworthy that Diamox® increased the U-P gradient to about the same extent as infusions of NaHCO<sub>3</sub> (Figure 3). This is more difficult to explain, inasmuch as the inhibition of renal carbonic anhydrase is associated with diminished overall H<sup>+</sup> secretion. It has recently been suggested that Diamox® elevates the U-P gradient by its buffering action rather than by its effect on carbonic anhydrase (3). If sufficient Diamox® is given, as appears to have been the case in the experiments just cited, its buffering action may be sufficient to produce these effects. This hypothesis, however, could hardly account for the present results. The amounts of Diamox® given were comparatively small (less than 1 mM), phosphate excretion did not appreciably increase, and titration curves disclosed no detectable rise in excretion of total urinary buffer.

Despite the fact that Diamox® depresses overall H<sup>+</sup> secretion, it is likely that accelerated production of H<sub>2</sub>CO<sub>3</sub> accounts for the high U-P gradients. If the major effect of Diamox® were on the proximal tubules with only minor inhibition exerted distally, then the increased amounts of NaHCO<sub>3</sub> which escaped proximal tubular reabsorption would be delivered to the distal tubular exchange site. Here, the Na<sup>+</sup>-H<sup>+</sup> exchange process would generate large amounts of H<sub>2</sub>CO<sub>3</sub> even though the maximum capacity of the distal tubular system to secrete H<sup>+</sup> might be somewhat depressed. In keeping with this explanation is the recent evidence suggesting that a major site of action of Diamox® is the proximal tubule (9).

If buffer excretion does not vary greatly, the magnitude of the U-P gradient appears to be determined by the rate of distal H<sup>+</sup> secretion relative to the rate of Na<sup>+</sup> reabsorption, as NaHCO<sub>3</sub> is delivered to the exchange site. From Figure 3, it is apparent that a maximum gradient of approximately 30 mm. Hg is established during water diuresis at a HCO<sub>3</sub><sup>-</sup> excretion rate of about 300 μEq. per minute. This represents the formation of at least 30 × 0.0309 or approximately 1 mM per L. H<sub>2</sub>CO<sub>3</sub> in excess of that generated in

urines with no gradient. In addition, of course, some  $\text{H}_2\text{CO}_3$  doubtless decomposes and is reabsorbed, so that the total distal  $\text{H}^+$  secretion would be much higher.

It is conceivable that Diamox® might generate high gradients by inhibiting red cell carbonic anhydrase. Inhibition of  $\text{CO}_2$  hydration in the red cell might elevate the  $\text{CO}_2$  tension of renal plasma and interstitial fluid. Urine equilibrating with these fluids would therefore have a high  $\text{CO}_2$  tension. This elevation of  $\text{pCO}_2$  would not be reflected in systemic blood since sufficient time would elapse in the passage of the blood to the heart and lungs to permit the hydration of  $\text{CO}_2$  via the uncatalyzed reaction to proceed to equilibrium. Thus, the calculation of the U-P gradient using the  $\text{CO}_2$  tension of arterial blood would give a falsely high value. Against such a mechanism, however, is the fact that renal blood flow is very high in relation to the rate of  $\text{CO}_2$  production by the kidney, so that inhibition of red cell carbonic anhydrase should have little effect on renal plasma  $\text{pCO}_2$ . Moreover, the relation between U-P and  $\text{HCO}_3^-$  excretion following the administration of Diamox® (Figure 3) is similar to that following  $\text{NaHCO}_3$  infusions, suggesting that the generation of  $\text{H}_2\text{CO}_3$  in the distal tubule is responsible for the U-P gradients in both instances.

#### SUMMARY

1. The relationship between bicarbonate diuresis and urine minus plasma (U-P)  $\text{pCO}_2$  gradient was examined in man during maximum  $\text{H}_2\text{O}$  diuresis.

2. It was found that during water diuresis, a maximum U-P  $\text{pCO}_2$  of about 30 mm. Hg developed at a  $\text{HCO}_3^-$  excretion rate of about 300  $\mu\text{Eq.}$  per minute. Buffer excretion was minimal during most of the experiments. The source of the  $\text{H}_2\text{CO}_3$  (subsequently dehydrated to  $\text{CO}_2$  and

$\text{H}_2\text{O}$ ) is most logically attributed to the secretion of  $\text{H}^+$  into luminal fluid containing  $\text{HCO}_3^-$ .

3. Marked increases in urine buffer excretion following phosphate infusions increased U-P gradients moderately, supporting the hypothesis that buffer can influence urinary  $\text{CO}_2$  tension by delaying the dehydration of  $\text{H}_2\text{CO}_3$ .

4. The high U-P gradients generated during Diamox® diuresis appear to be the consequence of  $\text{Na}^+ - \text{H}^+$  exchange as  $\text{NaHCO}_3$  floods the distal tubule, even though total  $\text{H}^+$  secretion at the distal tubular exchange site may be somewhat depressed. This suggests that the principal site of action of Diamox® is the proximal tubule.

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