Effect of phlorhizin on renal glucose and phosphate transport in the dog

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Summary

1. Clearance and micropuncture studies were performed in 19 thyroparathyroidectomized dogs to examine the inter-relationship between the renal transport of sodium, glucose and phosphate.

2. All experiments were carried out before and after the intravenous administration of phlorhizin [7 mg (15 μmol)/kg] with a sustaining infusion of the same dose/h. Thirteen dogs were studied during hydropenia (group I) and six dogs in the volume-expanded state (group II).

3. In the proximal tubule, phlorhizin significantly reduced sodium reabsorption in hydropenic dogs, but had no effect in volume-expanded dogs. Proximal tubular glucose reabsorption was completely inhibited by phlorhizin in both groups, but no significant change in phosphate reabsorption was observed.

4. Fractional glucose excretion in the urine reached 83–89% after phlorhizin, values significantly less than 100%, suggesting a residual reabsorption of glucose in a more distal segment or in deep nephrons. The changes in fractional excretion of sodium and phosphate were significantly correlated.

5. The effect of phlorhizin on both sodium and glucose reabsorption in the proximal tubule in hydropenic dogs suggests the existence of a cotransport mechanism, whereas the absence of an effect on sodium transport in volume-expanded dogs despite complete inhibition of glucose reabsorption indicates the existence of a sodium-independent component of net proximal tubular glucose transport.

6. Absence of the effect of phlorhizin on proximal tubular phosphate transport in the face of a significant reduction in sodium reabsorption implies that the reciprocal relationship between glucose and phosphate transport could be masked by the changes in sodium transport. Thus the sodium–phosphate transport relationship may prevail over that of glucose–phosphate in the proximal tubule.

Key words: glucose transport, kidney, micropuncture, phlorhizin, phosphate transport, sodium transport.

Abbreviations: GFR, glomerular filtration rate; PAH, p-aminohippuric acid.

Introduction

The renal transport of glucose and phosphate has been shown to be related to that of sodium (Robson, Strivastava & Bricker, 1968; Massry, Coburn & Kleeman, 1969; Suki, Martinez-Maldonado, Rouse & Terry, 1969; Kurtzman, White, Rogers & Flynn, 1972; Stolte, Hare & Boylan, 1972; Wen, 1974a; Wen, 1976a). Thus extracellular volume expansion and the administration of maleic acid or acetazolamide (Wen, 1974a; Wen, 1976a; Wen, Bynar & Stoll, 1978) inhibit the reabsorption of sodium, glucose and phosphate in the proximal tubule. On the other hand, there is also evidence for a reciprocal relationship between glucose and phosphate transport (Pitts & Alexander, 1944; Cohen, Berglund &
Lotspeich, 1956; Skeith, Healey & Cutter, 1970; Harter, Mercado, Rutherford, Rodriguez, Slatopolsky & Klahr, 1974; Dennis & Brazy, 1978), suggesting that they may compete for a common energy source for transport. In view of the uncertainty regarding the inter-relationship between the transport of these three substances by the kidney, we performed clearance and micropuncture studies in the dog after the administration of phlorhizin (Wen, 1974b), a glucoside known specifically to inhibit renal glucose transport (Frasch, Frohnert, Bode, Baumann & Kinne, 1970; Glossman & Neville, 1972; Silverman & Black, 1975). The use of such a specific inhibitor of glucose transport should provide information with regard to its concomitant effect on sodium and phosphate transport and should further elucidate the relationship between the transport of glucose, phosphate and sodium.

Methods

Clearance and micropuncture studies were performed in 19 mongrel dogs of both sexes, weighing 12–20 kg. The animals were fed pelleted dog food and had access to both food and water until the morning of the experiments. They were relatively hydropenic in that their mean urinary osmolality at the time of the studies was approximately 550 mosmol/kg. All animals were thyroparathyroidectomized about 2 h before the studies. The animals were divided into two groups according to the status of extracellular volume in the control phase.

In group I (13 dogs), micropuncture experiments were performed in the hydropenic state before and after the administration of phlorhizin. Fluid administration during the experiment in this group was limited to the administration of a 5% inulin solution at 1.1 ml/min. Phlorhizin (Phlorizin dihydrate; Accurate Chemical and Scientific Corp., Hicksville, NY, U.S.A.) was dissolved in Ringer’s solution in a concentration of 5 mg (11 μmol/ml), which required slight heating at 60°C in a water bath to facilitate dissolution. Phlorhizin was given intravenously in a dose of 7 mg (15 μmol/kg) followed by a sustaining infusion of the same dose/h. Clearance and micropuncture collections of samples were resumed for the second phase 30 min after phlorhizin administration began. In group II (six dogs), the experiments were performed in a sustained volume-expanded state before and after phlorhizin administration. Volume expansion was achieved by the infusion of Ringer’s solution at a rate of 24 ml/min until the expanded volume reached 10% of body weight. Thereafter a relatively constant extracellular volume was maintained by the continuous infusion of Ringer’s solution at a rate sufficient to replace the urinary loss of fluid. The mean sustaining infusion rate in group II was 4 ml/min. In each phase, three to five clearance collections were made with each collection lasting 15 min. Blood samples were drawn from the femoral artery at the mid-point of each collection period.

The animals were prepared for micropuncture as previously described (Wen, 1974a). Under pento-barbital anaesthesia, an endotracheal tube was inserted and respiration maintained with a Harvard respirator (Harvard Apparatus Co., Mills, MA, U.S.A.). Jugular and foreleg veins were cannulated for the infusion of fluid and both femoral arteries were cannulated for blood sampling and monitoring blood pressure. The urinary bladder was exposed by a suprapubic incision and both ureters were catheterized with PE-160 polyethylene tubing. The left kidney was exposed for micropuncture by a flank incision, dissected free from perirenal tissue and immobilized in a Lucite holder. The main renal artery of the left kidney was cannulated with a 27-gauge needle connected to PE-20 tubing for injection of lissamine green dye. The renal vein of the exposed kidney was cannulated with a 25-gauge needle for sampling renal venous blood. A small area (about 1 cm²) of renal capsule was carefully removed under direct vision through a dissecting microscope to expose the surface tubules. The kidney surface was covered with warmed mineral oil to prevent evaporation.

The recollection micropuncture technique was used to obtain proximal tubular fluid samples at the same sites in the two phases. Four to six proximal tubular fluid samples were obtained for each phase of the experiments. Late or the last accessible segments of the proximal tubule were selected for micropuncture by injecting 5% lissamine green (0.05 ml) into the renal artery and timing the appearance of the dye in the surface tubules. Injection of the dye into the renal artery, as was carried out in these experiments, did not cause unilateral natriuresis nor did it interfere with the determination of inulin, glucose or phosphate in the tubular fluid and urine. A long oil block of at least four to five times the tubular diameter in length was maintained immediately distal to the micropuncture site to prevent retrograde collection and the tubular fluid collection was timed for the determination of tubular fluid flow rate.

Tubular fluid volume was determined by measur-
ing the length of the fluid column in a constant-bore microcapillary tube with a Gaertner measuring microscope (Gaertner Scientific Corporation, Chicago, IL, U.S.A.). The tubular fluid was analysed for inulin by a fluorimetric method (Vurek & Pegram, 1966), for glucose by a fluorimetric hexokinase method (Stoll & Wen, 1978) and for phosphate by a colorimetric method (Chen, Toribara & Warner, 1956). Inulin and p-aminohippuric acid (PAH) in plasma and urine were measured by Autoanalyzer methods (Harvey & Brothers, 1962; Steele, 1969), glucose by a spectrophotometric hexokinase method (Peterson & Young, 1969), and sodium and potassium by flame photometry. Phosphate concentration in the plasma ultrafiltrate and urine was determined by an Autoanalyzer method (Kraml, 1966). Plasma ultrafiltrate was made by centrifugation of plasma samples in a collodion bag under optimal pH and temperature control. The presence of glucose and inulin in the tubular fluid and urine samples did not interfere with the chemical determination of each other.

The validity of recollection micropuncture techniques for proximal tubular fluid/plasma inulin, glucose and phosphate in the control animals in our laboratory has previously been documented (Wen, Wong, Evanson, Lockhart & Dirks, 1973; Wen, 1974a; Wen, 1976a). A comparison of single nephron filtration rate for two consecutive control phases in 13 hydropenic dogs yielded a mean recollection/control ratio of 1.06 ± SD 0.12, and in 12 volume-expanded dogs the ratio was 1.12 ± SD 0.19. Although these values showed a tendency to rise during recollection, the differences did not achieve statistical significance.

For the statistical analysis of the clearance and micropuncture data, the mean values for each phase of the experiment were compared by using the paired or unpaired Student’s t-test. A linear regression line and correlation coefficient were obtained to evaluate the relationship between the changes in the transport of two substances. To eliminate the effect of the transport of the third substance on the relationship between the other two, the partial correlation coefficient was calculated, the change in the transport of the third substance being kept constant (Steel & Torrie, 1960).

**Results**

The clearance data before and after the administration of phlorhizin in the two groups are summarized in Table 1. There were no significant changes in packed-cell volume, mean arterial blood pressure and glomerular filtration rate (GFR) in either group after the administration of phlorhizin. The volume expansion in group II was reflected in the lower values for packed-cell volume. Renal plasma flow was reduced significantly after phlorhizin in both groups, resulting in an increase in filtration fraction. In group I, urine flow increased from 0.7 to 1.4 ml/min after phlorhizin, but it did not increase significantly in group II, having already been increased by volume expansion to 3.6 ml/min. Plasma glucose concentration was lower in group II owing to haemodilution and fell significantly in both groups after phlorhizin owing to glycosuria. Absolute and fractional excretion of glucose increased dramatically after phlorhizin in both groups, reaching 83–89% of the filtered load. However, these values were significantly less than 100%, indicating incomplete inhibition of renal glucose reabsorption by phlorhizin at the dose employed in these studies.

Plasma ultrafiltrate phosphate was relatively unchanged after phlorhizin in both groups. In group I, the absolute and fractional excretion of phosphate rose slightly, but not significantly after phlorhizin, and the absolute and fractional excretion of sodium and potassium increased significantly. In Fig. 1, the changes in fractional excretion of phosphate in group I are plotted against the corresponding changes in sodium. There is a highly significant correlation \( P < 0.001 \) between the values for sodium and phosphate with a regression line of \( y = 5.05x - 3.52 \) and a correlation coefficient of 0.80. This indicates a close relationship between sodium and phosphate transport, which might have masked the relationship between glucose and phosphate transport. Therefore, the partial correlation coefficient between the changes in fractional excretion of glucose and phosphate with a fixed change in fractional sodium excretion was calculated. It yielded an r value of \(-0.65\), which is significant \( P < 0.02 \). Therefore the alteration in sodium transport significantly altered the reciprocal relationship between glucose and phosphate transport. In group II, the absolute and fractional excretion of sodium tended to increase further after the administration of phlorhizin in the volume-expanded state, but the increase was not statistically significant. Similarly, the slight reduction in fractional excretion of phosphate after phlorhizin was not significant.

Micropuncture data of studies on the late proximal tubule are summarized in Table 2. Single-nephron filtration rate did not change significantly
TABLE 1. Clearance data in hydropenic and volume-expanded dogs before and after the administration of phlorhizin

Values are means ± SEM for studies on the micropunctured kidney, when applicable. * P < 0.05; ** P < 0.01. Numbers in parentheses denote the numbers of dogs.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Experimental phase</th>
<th>Packed cell volume (%)</th>
<th>Blood pressure (mmHg)</th>
<th>GFR (ml/min)</th>
<th>Renal plasma flow (ml/min)</th>
<th>Urine flow (ml/min)</th>
<th>Plasma glucose (mmol/l)</th>
<th>Absolute glucose excretion (μmol/min)</th>
<th>Fractional glucose excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (13)</td>
<td>Hydropenia</td>
<td>44</td>
<td>132</td>
<td>30-7</td>
<td>92.5</td>
<td>0.70</td>
<td>5.80</td>
<td>0.70</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1</td>
<td>± 1 ± 1.9</td>
<td>± 11.7</td>
<td>± 0.17</td>
<td>± 0.20</td>
<td>± 0.06</td>
<td>± 0.06</td>
<td>± 0.05</td>
</tr>
<tr>
<td></td>
<td>Phlorhizin</td>
<td>43</td>
<td>132</td>
<td>30-1</td>
<td>71-3**</td>
<td>1.41**</td>
<td>4.37**</td>
<td>113.46**</td>
<td>88.88**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 1</td>
<td>± 4 ± 2.6</td>
<td>± 8.2</td>
<td>± 0.26</td>
<td>± 0.32</td>
<td>± 9.65</td>
<td>± 1.44</td>
<td></td>
</tr>
</tbody>
</table>

| Group II (6)       | Volume expansion  | 33                     | 127                    | 29.8         | 117.3                     | 3.59                | 4.93                    | 0.52                           | 0.37                          |
|                    |                   | ± 2                    | ± 5 ± 3.4              | ± 12.2       | ± 1.33                    | ± 0.46              | ± 0.09                  | ± 0.04                         |                               |
|                    | Phlorhizin        | 34                     | 127                    | 28.4         | 96.3*                     | 4.13                | 4.23*                   | 99.60**                        | 83.45**                       |
|                    |                   | ± 2                    | ± 7 ± 3.6              | ± 15.5       | ± 1.31                    | ± 0.45              | ± 16.87                 | ± 4.39                         |                               |

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Experimental phase</th>
<th>Plasma ultrafiltrate PO₄ (mmol/l)</th>
<th>Absolute PO₄ excretion (μmol/min)</th>
<th>Fractional PO₄ excretion (%)</th>
<th>Absolute Na excretion (μmol/min)</th>
<th>Fractional Na excretion (%)</th>
<th>Absolute K excretion (μmol/min)</th>
<th>Fractional K excretion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (13)</td>
<td>Hydropenia</td>
<td>2.29</td>
<td>3.77</td>
<td>5.02</td>
<td>58.4</td>
<td>1.21</td>
<td>28.5</td>
<td>26.8</td>
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<tr>
<td></td>
<td></td>
<td>± 0.08</td>
<td>± 0.94</td>
<td>± 1.16</td>
<td>± 10.2</td>
<td>± 0.19</td>
<td>± 2.3</td>
<td>± 1.2</td>
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<tr>
<td></td>
<td>Phlorhizin</td>
<td>2.44</td>
<td>4.67</td>
<td>5.71</td>
<td>97.8*</td>
<td>2.04*</td>
<td>42.5**</td>
<td>37.7**</td>
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<tr>
<td></td>
<td></td>
<td>± 0.14</td>
<td>± 1.68</td>
<td>± 1.56</td>
<td>± 23.0</td>
<td>± 0.32</td>
<td>± 4.1</td>
<td>± 1.7</td>
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<tr>
<td>Group II (6)</td>
<td>Volume expansion</td>
<td>1.98</td>
<td>7.05</td>
<td>11.13</td>
<td>290.2</td>
<td>5.90</td>
<td>43.7</td>
<td>47.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.19</td>
<td>± 2.11</td>
<td>± 1.82</td>
<td>± 119.6</td>
<td>± 1.90</td>
<td>± 8.0</td>
<td>± 6.2</td>
</tr>
<tr>
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<td>Phlorhizin</td>
<td>2.21</td>
<td>5.71</td>
<td>8.57</td>
<td>374.0</td>
<td>7.36</td>
<td>49.1</td>
<td>49.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>± 0.18</td>
<td>± 1.24</td>
<td>± 1.31</td>
<td>± 141.2</td>
<td>± 2.19</td>
<td>± 7.7</td>
<td>± 4.5</td>
</tr>
</tbody>
</table>

Fig. 1. Correlation between the changes in fractional excretion of phosphate and sodium in hydropenic dogs after the administration of phlorhizin. The regression line $y = 5.05x - 3.52$ had a correlation coefficient ($r$) of 0.80, which is highly significant ($P < 0.001$).

in either group. Mean proximal tubular fluid inulin/plasma inulin ratios for each experiment before and after phlorhizin are plotted in Fig. 2. In group I, all but one of the points are scattered below the line of identity, indicating inhibition of fractional fluid and sodium reabsorption in the proximal tubule. In group II, however, the points are clustered along the identity line, showing no effect of phlorhizin on sodium reabsorption in the proximal tubule when this was already inhibited by volume expansion. The effect of phlorhizin on sodium transport in group I was modest, amounting to about 6% of the filtered load (Table 2). Phlorhizin completely inhibited proximal tubular glucose reabsorption in both groups, with the unreabsorbed fraction reaching 105 and 99% respectively, values not significantly different from 100%. Absolute glucose reabsorption in the proximal convoluted tubule was reduced from 318 ± 22 to 14 ± 7 pmol/min in group I and from 246 ± 31 to 6 ± 13 pmol/min in group II. The corresponding values for sodium fell from 3.58 ± 0.28 to 2.96 ± 0.31 nmol/min in group I but remained unchanged at 2.49 ± 0.35 and 2.18 ± 0.48 nmol/min respectively in group II. The magnitude of phlorhizin inhibition of proximal tubular glucose reabsorption was 90% of the filtered load in group I and 65% in group II, with about 20% inhibition accounted for by volume expansion (Table 2). Micropuncture data on proximal tubular
TABLE 2. Micropuncture data of the late proximal tubule before and after the administration of phlorhizin

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Experimental phase</th>
<th>Single nephron GFR (nl/min)</th>
<th>Tubular fluid inulin/plasma inulin</th>
<th>Fractional Na delivery (%)</th>
<th>Tubular fluid glucose/plasma glucose</th>
<th>Fractional glucose delivery (%)</th>
<th>Tubular fluid PO₄/plasma ultrafiltrate PO₄</th>
<th>Fractional PO₄ delivery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (13)</td>
<td>Hydropenia</td>
<td>64.7 ± 4.2 1.60 ± 0.04</td>
<td>63.1 ± 1.6 0.23 ± 0.03</td>
<td>15.0 ± 1.7 0.03 ± 0.04</td>
<td>0.63 ± 0.03 0.03 ± 0.04</td>
<td>40.3 ± 2.0</td>
<td></td>
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<tr>
<td></td>
<td>Phlorhizin</td>
<td>61.7 ± 5.1 1.46**</td>
<td>68.9** ± 1.5 ± 0.04</td>
<td>1.50** ± 2.4 0.04 ± 0.04</td>
<td>0.60 ± 0.04 0.04 ± 0.04</td>
<td>41.3 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II (6)</td>
<td>Volume expansion</td>
<td>74.7 ± 5.9 1.30 ± 0.06</td>
<td>77.8 ± 3.4 0.34 ± 0.02</td>
<td>34.0 ± 2.4 0.03 ± 0.04</td>
<td>0.83 ± 0.03 0.03 ± 0.04</td>
<td>66.3 ± 3.1</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Phlorhizin</td>
<td>67.8 ± 9.2 1.34 ± 0.09</td>
<td>76.7 ± 5.1 0.12 ± 0.04</td>
<td>4.5 ± 0.04 0.04 ± 0.04</td>
<td>0.79 ± 0.04 0.04 ± 0.04</td>
<td>62.3 ± 5.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig 2. Effect of phlorhizin on proximal tubular fluid inulin/plasma inulin ratios in hydropenic and volume-expanded dogs. Each point represents the mean for each dog. Note that most points lie below the diagonal line of identity in the studies on hydropenic dogs (left) and along the line of identity in the studies on volume-expanded dogs (right).

Phlorhizin on glucose and phosphate transport are also shown in Table 2. Proximal tubular fluid phosphate/plasma ultrafiltrate phosphate ratios were about 0.6 and 0.8 in the hydropenic and volume-expanded groups respectively, and remained unchanged after phlorhizin in both groups. In group I, the fractional delivery of phosphate to the late proximal tubule was unchanged after phlorhizin (P > 0.7) despite a significant reduction in sodium reabsorption. The partial correlation coefficient between the changes in fractional proximal reabsorption of glucose and phosphate with a fixed change in fractional sodium reabsorption was -0.61, which is significant (P < 0.05). Thus the reciprocal relationship between proximal tubular glucose and phosphate transport appeared to be masked by the reduction in sodium transport. In group II, the fractional deliveries of both sodium and phosphate were high owing to the inhibitory effect of volume expansion on proximal tubular reabsorption, but no effect of phlorhizin was observed for sodium (P > 0.7) or phosphate (P > 0.3) when it was administered in the volume-expanded state.

Discussion

Our micropuncture studies in the dog have demonstrated that proximal tubular glucose reabsorption was completely inhibited by the administration of phlorhizin. A similar observation was made in the early work of Walker, Bott, Oliver & MacDowell (1941) in the rat and guinea pig by comparing the values of proximal tubular fluid/plasma ratios for glucose and creatinine. A number of clearance studies in man (Chasis, Jolliffe & Smith, 1933; Shannon & Smith, 1935) and the dog (Shannon, 1935) also indicate that phlorhizin completely inhibits total renal glucose reabsorption when given in large doses of 100–200 mg (212–424 μmol/kg). Our micropuncture studies showed that complete inhibition of glucose reabsorption in the proximal
convoluted tubule can be achieved at a dose of 7 mg (15 μmol)/kg, plus a sustaining infusion.

In our studies in hydropenic dogs, complete inhibition of proximal tubular glucose reabsorption was associated with a significant reduction in sodium transport in this segment. The exact mechanism of the effect of phlorhizin on sodium transport is unknown. PAH clearance decreased without a significant reduction in GFR, indicating an increase in filtration fraction. The associated change in peritubular capillary osmotic pressure should enhance rather than decrease proximal tubular sodium reabsorption (Lewy & Windhager, 1968). The fall in PAH clearance could in part have been related to the inhibitory effect of phlorhizin on PAH secretion (Smith, 1955; Braun, Whittaker & Lotspeich, 1957). It is not known whether the small increment in proximal tubular osmolality of about 5 mosmol/kg, estimated from the rise in proximal tubular fluid glucose/plasma glucose ratio, was sufficient to cause a reduction in sodium reabsorption. However, such an effect cannot be excluded since Holzgreve & Schrier (1975) showed that a difference of 10 mosmol/kg in the osmolality of peritubular capillary perfusates could cause a substantial change in proximal tubular sodium reabsorption. On the other hand, as phlorhizin is a specific inhibitor of renal glucose transport at the brush-border membrane (Frasch et al., 1970; Glossman & Neville, 1972; Silverman & Black, 1975), it is more reasonable to assume that its effect on sodium transport was secondary to that on glucose transport. It has been suggested that glucose and sodium share a common carrier for transport across the intestinal mucosal membrane (Crane, 1965) and a similar cotransport system has also been postulated at the brush-border membrane of the renal proximal tubule (Ullrich, 1976), which could be the basis for the reported correlation between glucose and sodium transport (Robson et al., 1968; Kurtzman et al., 1972; Stolte et al., 1972; Wen, 1976a; Weinman, Suki & Eknoyan, 1976). Our present observation showing inhibition of proximal tubular sodium reabsorption by a specific inhibitor of glucose transport is consistent with the concept of such a cotransport system.

Extracellular volume expansion inhibited sodium and glucose transport in the proximal tubule, confirming our previous observations (Wen, 1976a). When phlorhizin was administered to volume-expanded dogs, no effect on proximal tubular sodium reabsorption could be demonstrated. Table 2 provides some quantitative information on the shared transport between sodium and glucose. Of the total net glucose reabsorption in the proximal tubule, less than a quarter was inhibited by volume expansion, while phlorhizin completely inhibited the remainder of glucose reabsorption without an additional effect on sodium transport. This indicates that only a quarter of net glucose transport was shared by sodium transport, three-quarters being unrelated to sodium reabsorption. The existence of a sodium-independent glucose-transport system was also supported by our observation in another series of micropuncture studies in which proximal tubular glucose reabsorption increased, whereas sodium reabsorption was reduced during sub-threshold glucose loading (Wen, 1976b). These observations strongly support the concept that glucose reabsorption in the proximal tubule occurs both with and without concomitant net sodium reabsorption.

It was also noted in our studies that fractional glucose excretion in the urine after phlorhizin was significantly less than 100%, and complete inhibition of glucose reabsorption was observed in the proximal tubule. This was probably related to the submaximal phlorhizin dosage employed in our studies, as 100% fractional glucose excretion had been reported with a dose of 100–200 mg (212–424 μmol)/kg (Shannon, 1935). The incomplete inhibition of glucose reabsorption by phlorhizin could have occurred either in the proximal tubule of the deep nephrons or in the segments more distal to the micropuncture sites. Our own micropuncture studies on the distal tubule indicated that significant reabsorption of glucose occurred in the segment between the late proximal tubule and the distal tubule (Friedman, Stoll, Boynar & Wen, 1978); Frohnert, Höhmann, Zwiebel & Baumann (1970) suggested a possible glucose reabsorptive site in the collecting duct.

While the effect of phlorhizin on renal glucose transport was dramatic, its effect on phosphate transport was less clear in our studies. Phlorhizin had no significant effect on the fractional excretion of phosphate but nearly completely inhibited glucose reabsorption. It is noteworthy that the changes in fractional phosphate excretion correlated significantly with those in fractional sodium excretion, and the latter changes could have masked the effect of phlorhizin on phosphate transport. Such a masking effect of the changes in sodium transport on the reciprocal relationship between glucose and phosphate transport was supported by the significant partial correlation obtained between the changes in fractional glucose
and phosphate excretion when the change in fractional sodium excretion was assumed constant. Similarly, the significant reduction in sodium reabsorption in the proximal tubule after phlorhizin in hydropenic dogs was associated with an unchanged phosphate transport. This observation is significant because a reduction in sodium reabsorption in the proximal tubule is normally associated with a parallel reduction in phosphate reabsorption under a variety of experimental conditions (Wen, 1974a; Wen, 1976a). It is possible, therefore, that the inhibition of glucose reabsorption by phlorhizin enhanced phosphate reabsorption, but this was offset by the effect of reduced sodium reabsorption on phosphate transport. This was also supported by the significant partial correlation between the changes in fractional proximal tubular glucose and phosphate reabsorption for a fixed change in sodium transport.

The absence of a direct effect of phlorhizin on the fractional excretion of phosphate in our studies was somewhat surprising because an enhancement of phosphate reabsorption by phlorhizin is well documented (Pitts & Alexander, 1944; Cohen et al., 1956; Skeith et al., 1970; Harter et al., 1974; Dennis & Brazy, 1978). The reciprocal relationship between glucose and phosphate transport was thought to be due to their competition for a common energy source for reabsorption rather than a common carrier for transport (Pitts & Alexander, 1944). However, both glucose and phosphate transport are known to be related to that of sodium (Robson et al., 1968; Kurtzman et al., 1972; Wen, 1974a; Massry et al., 1969; Suki et al., 1969; Wen, 1976a; Ullrich, 1976; Hoffmann, Thees & Kinne, 1976). There are a number of experimental conditions in which alterations in the transport of sodium, glucose and phosphate occur in the same direction (Wen, 1974a; Wen, 1976a; Wen et al., 1978). Also, a sodium-independent phosphate transport in the proximal tubule has been demonstrated (Wen, 1974a; DeFronzo, Goldberg & Agus, 1976). Thus there appears to be two components in the transport of glucose and phosphate: one component being sodium-dependent, which leads to parallel changes in glucose and phosphate along with sodium transport, the other being sodium-independent, which could be responsible for the reciprocal relationship between glucose and phosphate transport. The exact manner by which these components of glucose and phosphate transport contribute to their net transport remains to be elucidated.

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