EFFECTS OF ETHACRYNIC ACID AND FRUSEMIDE ON URINARY PHOSPHATE IN THE DOG

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SUMMARY

1. The renal excretion of phosphate was studied in dogs under control conditions and during infusion of ethacrynic acid or frusemide at different levels of filtered load.

2. Both of these diuretic compounds significantly enhanced the fractional excretion of phosphate at normal and moderately decreased filtered loads, and a good correlation between fractional excretions of sodium and phosphate could then be demonstrated.

3. When the blood flow to the left kidney was decreased by constricting the aorta below the right and above the left renal arteries, fractional excretion of phosphate decreased below control levels in the left kidney although that of sodium remained above pre-diuresis values.

4. The phosphaturic effect of ethacrynic acid and frusemide might be the result of a proximal site of action. The reversal of the phosphaturia by aortic constriction might have been caused by enhanced proximal reabsorption of phosphate and minimal delivery of this ion to the more distal sites of diuretic action.

Key words: aortic constriction, diuretics, filtered load, phosphaturia.

The results of studies of the effects of ethacrynic acid and frusemide on renal excretion of phosphate have been inconclusive. Oral and intravenous administration of ethacrynic acid caused a decrease in urinary phosphate excretion, and this effect was related to a possible decrease in parathyroid hormone secretion (Demartini, Briscoe & Ragan, 1967). The renal clearance of phosphate did not change in dogs infused with a low dose of ethacrynic acid or frusemide (Duarte, 1968), but when each of the two diuretics was given to human volunteers, only frusemide caused phosphaturia (Puschett & Goldberg, 1968). In thyroparathyroid-ectomized dogs, the administration of several diuretics, including ethacrynic acid and frusemide, caused an increase in fractional excretion of phosphate, from which it was deduced that previous conflicting reports might have been caused by changes in parathyroid hormone secretion (Eknoyan, Suki & Martinez-Maldonado, 1970). Finally, in human volunteers given...
ethacrynic acid or frusemide, enhanced excretion or retention of phosphate has been observed, depending on variations in plasma volume and glomerular filtration rate (Steele, 1971). Here we report studies designed to clarify and evaluate further the effects of ethacrynic acid and frusemide on the renal handling of phosphate in the dog. Also, an effort was made to study the relationship between the renal handling of phosphate and sodium during the infusion of both diuretics at normal rates of glomerular filtration and during acute reduction in filtered load to the left kidney by aortic constriction.

**METHODS**

Experiments were performed in male and female mongrel dogs (body weight, 17–27 kg) anaesthetized with 2.5% thiopental. Catheters were inserted in the forelegs for infusion of fluid, in a jugular vein for sampling of blood, and in each ureter for urine collection from the right and left kidneys. A subcostal incision was made in the left flank, the retroperitoneal space was entered by blunt dissection of the muscles, and an umbilical tape was placed around the aorta below the right renal artery and above the left renal artery.

![Experimental design](image)

Fig. 1. Experimental design. Broken lines indicate equilibration periods. For details see the text.

The experimental design is illustrated in Fig. 1. A diuresis was induced by the intravenous administration of 154 mmol/l sodium chloride; after appropriate priming, a sustaining infusion of inulin was started, and 30 min was allowed to elapse for equilibration. In all experiments (ten dogs) collection of urine was made in three 10-min control periods, and then either ethacrynic acid (five dogs) or frusemide (five dogs) was administered intravenously. The priming dose of ethacrynic acid was 16.5 μmol/kg (5 mg/kg) and of frusemide 15 μmol/kg (5 mg/kg), and the same amounts were infused hourly at a constant rate throughout the duration of the experiments. This was followed by four 15-min collections under each of the following experimental conditions. Diuresis period 1: the blood flow to the kidneys was not disturbed; period 2: the filtered load to the left kidney was decreased by 50% by partial constriction of the aorta; period 3: studies were made after complete release of the partial aortic constriction. Equilibration periods, each of 15-min duration, elapsed before the initiation of
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each diuresis period; thus urine collections were made under steady-state conditions, when the urine flow from each kidney was equal and constant.

The urinary losses were replaced by intravenous administration of Ringer's solution (Na, 130 mmol/l; K, 4 mmol/l; Ca, 1.5 mmol/l; Cl, 109 mmol/l; lactate, 28 mmol/l; Mg, 0.75 mmol/l) in a volume equal to the urine collected.

Inulin was determined in plasma and urine by the resorcinol method (Schreiner, 1950), and the clearance of inulin was used as a measure of glomerular filtration rate. Phosphate was measured by colorimetry (Fiske & Subbarow, 1925); sodium was measured by flame photometry. Total and ultrafilterable (Toribara, Terepka & Dewey, 1957) plasma calcium and magnesium and urinary calcium and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer 303). Haematocrit values were measured in each blood sample.

The results were analysed by standard statistical methods (Snedecor & Cochran, 1967).

RESULTS

Period 1: effects of ethacrynic acid and frusemide before aortic constriction

Ethacrynic acid caused a decrease in glomerular filtration rate, and in filtered load of phosphate, of approximately 30% (Table 1). Phosphate reabsorption decreased significantly and, although absolute excretion increased, the change was not statistically significant. The clear-

<table>
<thead>
<tr>
<th>Table 1. Renal effects of ethacrynic acid</th>
</tr>
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<tbody>
<tr>
<td>All values are mean ± SEM. ( \text{P}<em>1 ) = phosphate; ( C</em>{\text{inulin}} ) = inulin clearance; ( \text{FE}_p ) = fractional excretion of phosphate; ( \text{FE}_n_a ) = fractional excretion of sodium. Period 1 = before aortic constriction; period 2 = during partial aortic constriction; period 3 = after release of aortic constriction.</td>
</tr>
<tr>
<td>Measurement</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>( V ) (ml/min)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>( C_{\text{inulin}} ) (ml/min)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>( \text{P}_1 ) excreted (( \mu \text{mol/min} ))</td>
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<tr>
<td></td>
</tr>
<tr>
<td>( \text{FE}_p ) (%)</td>
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<td></td>
</tr>
<tr>
<td>( \text{Na} ) excreted (( \mu \text{mol/min} ))</td>
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<tr>
<td></td>
</tr>
<tr>
<td>( \text{FE}_n_a ) (%)</td>
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<td></td>
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</tbody>
</table>

\(^{(1)}\) For difference from control, \( P < 0.001. \)

\(^{(2)}\) For difference from control, \( P < 0.01. \)
ance of inulin decreased markedly whereas the clearance of phosphate remained close to control values, thus resulting in an increase in fractional excretion of phosphate. Sodium reabsorption decreased, and absolute and fractional excretions increased significantly.

In contrast, frusemide did not affect glomerular filtration rate, and filtered load remained constant (Table 2). It caused a significant increase in fractional and absolute excretions of phosphate and a decrease in reabsorption. Fractional and absolute excretions of sodium also increased significantly.

**Table 2. Renal effects of frusemide**

All values are mean±SEM. For abbreviations and explanation of Periods 1–3 see Table 1.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Kidney</th>
<th>Control</th>
<th>Frusemide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Period 1</td>
</tr>
<tr>
<td>V (ml/min)</td>
<td>R</td>
<td>1·41 ±0·21</td>
<td>12·21 ±0·97(1)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>1·42 ±0·18</td>
<td>12·41 ±1·13(1)</td>
</tr>
<tr>
<td>C\textsubscript{inulin} (ml/min)</td>
<td>R</td>
<td>36 ±2·40</td>
<td>35 ±2·27</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>34 ±2·81</td>
<td>35 ±2·74</td>
</tr>
<tr>
<td>P\textsubscript{f} excreted (µmol/min)</td>
<td>R</td>
<td>3·83 ±0·69</td>
<td>5·64 ±0·65(2)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>3·81 ±0·68</td>
<td>5·29 ±0·57</td>
</tr>
<tr>
<td>FE\textsubscript{P} (%)</td>
<td>R</td>
<td>21·97 ±2·19</td>
<td>35·45 ±2·47(1)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>21·12 ±1·39</td>
<td>31·94 ±2·32(1)</td>
</tr>
<tr>
<td>Na excreted (µmol/min)</td>
<td>R</td>
<td>250 ±19·98</td>
<td>1818 ±157·12(1)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>233 ±23·67</td>
<td>1873 ±158·89(1)</td>
</tr>
<tr>
<td>FE\textsubscript{Na} (%)</td>
<td>R</td>
<td>4·70 ±0·61</td>
<td>35·55 ±2·75(1)</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>5·44 ±0·67</td>
<td>33·91 ±1·82(1)</td>
</tr>
</tbody>
</table>

(1) For difference from control, $P<0·001$.
(2) For difference from control, $P<0·005$.
(3) For difference from control, $P<0·01$.

**Period 2: effects of ethacrynic acid and frusemide during aortic constriction**

During acute decrease in filtered load to the left kidney by aortic constriction, the effects of ethacrynic acid and frusemide were similar; glomerular filtration rate decreased by approximately 50%, compared with the same side in the diuretic periods before and after release of constriction and with the non-constricted kidney in the same periods. Fractional and absolute excretions of phosphate by the left kidney decreased markedly, to below pre-diuretic values; they remained increased in the right kidney. Although fractional and absolute excretions of sodium by the left kidney decreased, they still remained significantly above control values. As illustrated in Fig. 2, the natriuresis was more pronounced with ethacrynic acid, and frusemide had a more pronounced phosphaturic effect in collection periods from the right kidney (non-stenotic side).
Period 3: effects of ethacrynic acid and frusemide after release of the aortic constriction

In this portion of the studies, right renal diuretic effects were comparable with those described during diuresis periods 1 and 2. As the partial aortic constriction was removed, left filtered load increased, and left renal function was similar to that of the right kidney.

There were no significant changes in plasma concentrations of phosphate, in total and ultrafilterable plasma calcium and magnesium concentrations, or in blood haematocrit values in these experiments.

During aortic constriction, there was a good correlation between fractional excretions of sodium and phosphate in the right kidney. However, in the left kidney, where the filtered load was markedly decreased, the correlation was poor (Figs. 2a and 2b).

**DISCUSSION**

Ethacrynic acid (Goldberg, McCurdy, Foltz & Bluemle, 1964) and frusemide (Suki, Rector & Seldin, 1965) interfere with the mechanism of urinary concentration and dilution, and their main site of action has been localized on the ascending portion of Henle's loop. Micropuncture studies on water and sodium reabsorption have demonstrated an effect of both diuretics in the proximal tubule when plasma volume and filtered load are maintained constant (Clapp, Nottebohm & Robinson, 1971; Burke, Robinson & Clapp, 1972). However, this effect was abolished when glomerular filtration rate decreased moderately and was reversed when the filtered load was decreased markedly (Knox, Wright, Howards & Berliner, 1969; Clapp et al., 1971; Burke et al., 1972).
Micropuncture and microinjection studies in the rat indicate that phosphate reabsorption is restricted to the proximal tubule (Strickler, Thompson, Klose & Giebisch, 1964; Murayama, Morel & Le Grimellec, 1972; Staum, Hamburger & Goldberg, 1972; Brunette, Taleb & Carriere, 1973). This conclusion is of great importance because the effects on urinary phosphate have been taken to indicate a proximal site of action of diuretics on sodium reabsorption (Puschett & Goldberg, 1968). Micropuncture studies reported by others, however, have demonstrated a significant contribution of the more distal portions of the nephron to the reabsorption of phosphate (Amiel, Kuntziger & Richet, 1970; Maesaka, Levitt & Abramson, 1973; Le Grimellec, Roinel & Morel, 1973). The problem remains unresolved at the moment (Knox, Schneider, Willis, Strandhoy & Ott, 1973).

Data from the literature suggest that there is a correlation in the renal handling of sodium and phosphate in the proximal tubule during different experimental conditions (Massry, Coburn & Kleeman, 1969; Suki, Martinez-Maldonado, Rouse & Terry, 1969; Agus, Puschett, Senesky & Goldberg, 1971; Fulop & Brazeau, 1971; Puschett, Agus, Senesky & Goldberg, 1972). However, the final changes in the urine indicate a dissociation in the distal tubule (Agus et al., 1971; Maesaka et al., 1973).

The results reported here indicate that, although ethacrynic acid and frusemide had similar diuretic action, there were differences in their effects on the renal handling of phosphate. Both caused an increase in fractional excretion of phosphate, but with frusemide there also was an increase in absolute excretion.

It is possible that the actions of ethacrynic acid and frusemide on tubular reabsorption of phosphate are mediated through an effect in the proximal tubule. During the administration of frusemide, the filtered load remained constant, and the phosphaturia might have been the result of a proximal inhibition in phosphate reabsorption. Because ethacrynic acid caused a moderate decrease in filtered load, the lack of a significant increase in absolute excretion of phosphate might have been related to a weaker proximal natriuretic effect. Consequently, the delivery of sodium out of the proximal tubule might have been less with ethacrynic acid. Because overall sodium excretion was slightly greater with ethacrynic acid, the natriuretic effect in the distal nephron must have been more pronounced with ethacrynic acid than with frusemide. Clearance studies have also demonstrated some differences in the diuretic effects of ethacrynic acid and frusemide related to different sites of action within the nephron (Lant, Baba & Wilson, 1967).

A good correlation between fractional excretions of sodium and phosphate in the right kidney during administration of ethacrynic acid and of frusemide supports the possibility of an interdependence in the transport mechanisms of both ions at normal (frusemide) and moderate decreases in (ethacrynic acid) filtered load. With more pronounced decreases in filtered load by aortic constriction, however, phosphate excretion seemed to have been fixed at a lower level and to be independent of sodium excretion with both diuretics. Aortic constriction might have caused a disruption in glomerulotubular balance in the left kidney, resulting in enhanced reabsorption of phosphate. Because the reabsorptive process for sodium takes place throughout the nephron, the continued natriuresis during aortic constriction and diuretic administration could have been the result of an inhibition in sodium reabsorption in the more distal portions of the nephron, at a site where phosphate reabsorption is believed to be insignificant or, at best, has been dramatically decreased by the fall in filtered load and the extensive reabsorption at the proximal tubule.
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REFERENCES


