Micropuncture study of renal tubular reabsorption of calcium in normal rodents

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CAICUM EXCRETION by the mammalian kidney has been extensively investigated by clearance techniques and is known to involve glomerular filtration, with subsequent reabsorption of most of the filtered calcium load (1). Available evidence indicates that calcium reabsorption involves active transport, but the nature of the reabsorptive process and its anatomic localization in the tubule remain unresolved. Recent stop-flow studies in dogs have been interpreted as showing active reabsorption of calcium in distal portions of the nephron (2, 3). No evidence of active calcium transport in more proximal parts of the nephron was found in these studies, but the stop-flow technique is of limited value in the study of proximal tubular function because it is impossible to control the degree to which the composition of proximal fluid is modified by continuing glomerular filtration during stop flow (4), or by passage through more distal parts of the nephron after release of the ureteral clamp.

On the other hand, the likelihood of proximal calcium reabsorption is suggested by other studies relating calcium to sodium excretion. Beck, Levitin, and Epstein (5) have presented evidence that hypercalcemia leads to impaired reabsorption of sodium by the renal tubules. Conversely, Walser (6) found in diuretic dogs that changes in sodium clearance were accompanied by parallel changes in calcium clearance and that, in fact, the clearance of free calcium ion equaled the sodium clearance. Since the bulk of sodium reabsorption occurs in the proximal tubule (7, 8), and this has been shown to involve active transport (9, 10), the above observations suggest that calcium also may be actively transported in the proximal tubule. We have investigated the renal tubular reabsorption of calcium in normal rodents, using the micropuncture technique to sample fluid in individual tubules. The results indicate that, as is true of sodium, calcium is actively reabsorbed from all parts of the nephron, and furthermore, that the bulk of calcium reabsorption occurs in the convoluted portion of the proximal tubule.

METHODS

White Rats

Normal rats were anesthetized by intraperitoneal injection of pentobarbital, 35 mg/kg body wt., the left kidney exposed through an abdominal incision, and 100–200 μC calcium 45 administered in a single intravenous injection through an indwelling jugular cannula. After allowing an hour or more for isotopic equilibration, fluid was collected by micropuncture from glomeruli and from proximal and distal convolutions on
TABLE I. Continued

<table>
<thead>
<tr>
<th>Rat</th>
<th>Proxi-</th>
<th>Calcu-</th>
<th>Dist-</th>
<th>Proxi-</th>
<th>Calcu-</th>
<th>Dist-</th>
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<td>Wt., g</td>
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<td>F/P of Tubule</td>
<td>mal % of Conv</td>
<td>F/P of Conv</td>
<td>mal % of Tubule</td>
<td>F/P of Tubule</td>
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</tr>
<tr>
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<td>72</td>
<td>.82</td>
<td>60</td>
<td>.82</td>
<td>49</td>
<td>.82</td>
</tr>
</tbody>
</table>

A. Nondiuretic rats

B. Mannitol diuresis rats

the surface of the kidney, as previously described (11, 12). To prevent retrograde flow, a droplet of mineral oil was injected into each tubule, and the rate of collection adjusted to maintain the oil at a position just distal to the puncture site. In this manner approximately 0.1 μl of tubular fluid could be collected in 20-60 min. Each sample was discharged under mineral oil into a siliconed glass dish and then aspirated into calibrated, constant-bore Pyrex capillary tubing, between layers of chloroform to prevent evaporation, and volume estimated by measurement of the length of the sample with an eyepiece micrometer. Samples were then discharged onto aluminum planchets, and after drying overnight at room temperature, radioactivity was determined in a windowless flow proportional counter.

![Figure 1](http://ajplegacy.physiology.org/)

**FIG. 1.** Calcium 45 fluid:plasma ratios in proximal tubule and distal convolution in 19 nondiuretic rats. Solid line through proximal points was fitted by the method of least squares. Broken line indicates average concentration ratio in glomerular filtrate.
TUBULAR REABSORPTION OF CALCIUM

FIG. 2. Calcium 45 fluid:plasma ratios in proximal tubule in 19 rats during mannitol diuresis. Shaded area indicates distribution of values in nondiuretic animals.

Similar measurements were also made on ureteral urine, and on plasma obtained from the inferior vena cava before and after each micropuncture. Self-absorption corrections were not necessary because of the small size of the samples. After the initial equilibration period, plasma radioactivity declined slowly in logarithmic fashion, with a half-disappearance time of 134 ± 2 hr. Similar studies were performed in normally hydrated, nondiuretic animals, and in rats undergoing osmotic diuresis induced by the intravenous infusion of 20% mannitol at 5 ml/hr. At the end of each experiment the kidney was removed and macerated, and puncture sites were localized by microdissection (11). As in previous studies, the proximal tubule is considered to extend from the glomerulus to the beginning of the thin descending limb of the loop of Henle; the distal convolution, from the macula densa to the point at which two or more convolutions join to form a collecting duct. Most of the rats were males of the Wistar strain, but several females, both Wistar and Sprague-Dawley, were also used. Weights varied from 110 to 460 g, with most animals being in the 200-300-g range.

Hamsters

Golden hamsters, 80-110 g in weight, were prepared in similar fashion, except that the upper part of the left ureter was opened to expose the tip of the renal papilla. Fifty to one hundred microcuries of Ca45 was injected intravenously, and after equilibration, fluid was collected by micropuncture from loops of Henle and from the orifices of terminal collecting ducts. Analyses were performed as noted above.

RESULTS

Nondiuretic Animals

Micropuncture studies were performed in 19 nondiuretic rats and 4 hamsters. Fluid:plasma Ca45 concentration ratios in the rat tubules are recorded in Table 1A. Localization of puncture sites is indicated as per cent of total length of tubule or convolution.

Proximal tubule. Fluid was collected from 4 glomeruli and 50 proximal tubules in 17 nondiuretic rats. The average fluid:plasma calcium concentration ratio was 0.71 in the four glomeruli and 0.76 in the proximal convolution. As is shown graphically in Fig. 1, the calcium concentration ratio remained nearly constant along the entire length of the convolution. The equation of the straight line fitted to the points by the method of least squares is \( y = 0.71 + 0.0012 x \). Although the calcium ratio appeared to rise slightly along the tubule, the increase is small and of questionable significance. The difference between the average calcium ratios in the first and last halves of the proximal convolution, 0.74 and 0.79, respectively, is not statistically significant (P > 0.10).

Loop of Henle. Samples were collected from nine loops of Henle at the tip of the renal papilla in four hamsters. The average fluid:plasma calcium ratio of these samples was 2.0 (range 1.3-2.4).

Distal convolution. Tubular fluid was collected from 27 distal convolutions in 14 nondiuretic rats. The average fluid:plasma calcium ratio of these samples was 0.47 (range 0.13-0.89). As shown in Fig. 1, the calcium concentration ratio varied widely among individual tubules, but in nearly all the samples it was lower than the concentration in proximal tubules or glomerular filtrate.

Ureteral urine. The average Ca45 concentration in 66 samples of ureteral urine from the 19 nondiuretic rats was 0.9 times the concentration in plasma. The U/P calcium ratio in 14 samples of collecting-duct urine from 4 nondiuretic hamsters averaged 1.2.

Osmotic Diuresis

Brisk osmotic diuresis was induced in 19 rats by intravenous infusion of 20% mannitol solution at the rate of 5.0 ml/hr. Micropuncture results are summarized in Table 1B. The average fluid:plasma calcium concentration ratio was 0.69 in 5 glomeruli, and 0.61 in 54 samples of proximal fluid. The lowest fluid:plasma ratio observed was 0.21, and as is shown graphically in Fig. 2, the ratio in 24 of the 54 tubules sampled was less than 0.60. In contrast, all but 1 of 30 proximal tubules in the nondiuretic rats had ratios greater than 0.60.

The calcium concentration in fluid from distal convolutions in the diuretic animals was very low, averaging only 0.07 X plasma concentration in 11 collections. This must be considered only an approximate estimate of the calcium concentration in these tubules, because the level of radioactivity in the samples was too low to permit adequate counting statistics. The calcium concentration in ureteral urine of the diuretic animals was also very low, averaging 0.04 X plasma concentration in 26 samples.
TABLE 2. Comparison of urinary excretion of Ca\textsuperscript{45} and stable Ca in rats

<table>
<thead>
<tr>
<th>Source</th>
<th>Calcium U/P Ratio</th>
<th>Chemical</th>
<th>Ca\textsuperscript{45}</th>
</tr>
</thead>
<tbody>
<tr>
<td>290-g Male, nondiuretic</td>
<td>0.41</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>260-g Male, nondiuretic</td>
<td>1.04</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td>220-g Female, saline diuresis</td>
<td>0.32</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>360-g Male, saline diuresis</td>
<td>0.41</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>0.18</td>
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</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.17</td>
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DISCUSSION

Specific Activity Studies

To test the validity of the radioisotope measurements as a true index of calcium excretion, simultaneous determinations of Ca\textsuperscript{45} and stable calcium were performed on urine and plasma obtained from two nondiuretic rats and from two in which the rates of urine formation and calcium excretion were increased by infusion of 5% NaCl, in order to shorten urine-collection times and to reduce dead-space errors. Collections were begun 1 hr after administration of Ca\textsuperscript{45}, thus duplicating conditions in the micropuncture experiments. Stable calcium was determined by flame photometry (13) or by the microspectrophotometric method of Webster (14). Urine: plasma concentration ratios for Ca\textsuperscript{45} and stable calcium are shown in Table 2. Although there was considerable variation among different animals in the ratio of urine to plasma calcium, in each instance the ratio computed from radioisotope measurements was identical to that calculated from the stable calcium determinations. These results in rats are in agreement with similar observations in dogs reported several years ago by Govaerts (15), and they indicate that under the conditions of these experiments the radioisotope measurements provide a reliable index of calcium excretion.

Approximately 70% of plasma calcium was found in this study to be filtrable at the glomerulus. The degree of protein binding of calcium in rat plasma has not been previously investigated, but our data are compatible with studies in dogs (16) and humans (17), which indicate that at physiological temperature and pH, 60-70% of plasma calcium is ultrafiltrable. Our results further demonstrate that although there is net trans-tubular loss of calcium out of all parts of the nephron, the bulk of calcium reabsorption normally occurs in the convoluted portion of the proximal tubule. The average calcium concentrations at various points along the rat nephron, expressed as a fraction of the concentration in glomerular filtrate, are recorded in the first column of Table 3. Average concentration ratios for inulin-C\textsuperscript{14}OOH at the same levels, obtained in a comparable group of animals (8), are noted in the second column. As is shown in the third column of the table, the calcium ratio divided by the inulin ratio at any point in the nephron is a measure of the quantity of calcium reaching that point relative to the amount filtered at the glomerulus. Thus it is seen that net reabsorption of calcium equivalent to approximately two-thirds of the filtered load occurs in the proximal convolution, 20-25% in the loop of Henle, and 10% in the distal convolution, with most of the small amount remaining at the end of the distal convolution being reabsorbed in the collecting ducts. This demonstration that the bulk of calcium reabsorption occurs in the proximal convolution is consistent with other observations on the function of this part of the nephron, which has been previously shown to be the major site of reabsorption of water, sodium, chloride, and bicarbonate (7, 8, 18).

Calcium loss in the loop of Henle appears to take place primarily from the ascending limb. Although one cannot, of course, make precise quantitative comparisons between fluid from long loops of juxtamedullary nephrons in the hamster and surface tubules in the rat, the high calcium concentration found at the bends of these loops points to the ascending limb as the important site of calcium reabsorption in the loop of Henle. Thus the major site of calcium loss in the loop appears to coincide with the locus of sodium transport (8).

Although in nondiuretic animals no decrease in calcium concentration occurs in the proximal convolution, the results in mannitol diuresis indicate active transport of calcium in this segment. If water reabsorption is restricted by the presence of nonreabsorbable solute, the tubular concentration of calcium falls. As noted above, under these conditions we have observed calcium concentrations in the proximal tubule as low as 0.21 X plasma concentration. Since the lumen of the proximal tubule has been shown to be negatively charged with respect to its exterior (19, 20), calcium ion under these conditions is being reabsorbed against both an electrical and a chemical gradient, and hence by an active process. Similar observations have been made with respect to sodium in the proximal tubule (9, 10). In nondiuretic animals, sodium with its attendant anions forms the bulk of osmotically active solute in proximal fluid, and as sodium is reabsorbed no measurable con-
concentration change occurs because of the simultaneous reabsorption of water. On the other hand, when water reabsorption from the tubule is limited, as in mannitol diuresis, the sodium concentration of proximal fluid falls, and active transport can be demonstrated.

The low calcium concentration found in nondiuretic animals along the entire length of the distal convolution, in the face of continuing water loss, indicates active transport of calcium out of this segment, as well as from the ascending limb of the loop of Henle. This is further supported by the very low calcium concentration found in the distal convolution during mannitol diuresis. Further reabsorption of calcium occurs from the collecting ducts, but it is not possible from our data to determine whether or not this involves active transport. Since sodium is actively transported at this locus, however, we feel that it is probable that calcium is actively transported here as well.

It is apparent from the foregoing discussion that the pattern of tubular reabsorption of calcium bears many similarities to sodium reabsorption. Walser (6) has observed in diuretic dogs that the clearance of free calcium ion equals the sodium clearance in states of varying urine flow and sodium excretion, provided urine flow exceeds 2.5 ml/min. Our results indicate that this relationship also holds true at the various levels in individual renal tubules in nondiuretic rodents. In Table 4 average calcium and sodium concentrations in the various segments of the nephron are expressed as a fraction of the concentrations in glomerular filtrate. The sodium values are derived from previously reported data (10, 21). The calcium ratios are qualitatively similar to the sodium ratios at every level except in the final urine, and the ratios are in close quantitative agreement in the proximal and distal convolutions. The relatively high concentration of calcium in the final urine is probably due to the presence of large amounts of anions which complex calcium, such as phosphate, citrate, and sulfate, in the very concentrated urine of these nondiuretic animals, as suggested by Walser. The apparent disagreement between the calcium and sodium ratios in the loop of Henle is of much smaller magnitude, and may be due to the small number of determinations on which the comparison is based. Both calcium and sodium concentrations in loop fluid should vary with the osmolality of the fluid, and the two groups of hamsters may not have been entirely comparable in this respect. Although definitive proof is lacking, our results are consistent with Walser's speculative hypothesis that sodium and calcium ions are in competition for a common binding site at the cell membrane.

REFERENCES