Inorganic Phosphate Homeostasis

RENAL ADAPTATION TO THE DIETARY INTAKE IN INTACT
AND THYROPARATHYROIDECTOMIZED RATS

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ABSTRACT The possibility of renal tubular adaptation to variations in dietary inorganic phosphate (Pi) was investigated in intact and thyroparathyroidectomized (TPTX) rats pair-fed diets containing low, normal, and high amounts of Pi for periods up to 10 days. Clearances were measured before and during acute i.v. infusions with Pi in conscious animals. Thus tubular reabsorption of phosphate (TRPi) could be assessed over a wide range of plasma phosphate concentrations ([Pi]pl). It was found that the renal tubule could adapt its capacity to transport Pi according to the dietary Pi: TRPi was always higher, for a given [Pi]pl, in the animals fed low than in those fed higher Pi diets. This diet-induced modification also occurred in the absence of thyroparathyroid glands, in the presence of the same calcemia and urinary pH, and during marked extracellular volume expansion. A time-course study in rats TPTX both before and during the administration of the experimental diets showed that a difference in the tubular handling of Pi was detectable as early as 3 days after switching the animals from a normal to low- or high-Pi diets. These results indicate that factors other than parathyroid hormone are implicated in the tubular response to variations in the dietary intake of inorganic phosphate.

INTRODUCTION

The renal response to a variation in the dietary intake of inorganic phosphate (Pi) has been relatively neglected in the last decade compared with the study of other factors that influence the excretion of this anion, such as parathyroid hormone (PTH) and other hormones, extracellular volume expansion (ECVE), calcium, and magnesium. It is well established that the urinary excretion of Pi is related to its dietary intake. This relation has been described by balance and clearance techniques in normal human adults and patients with parathyroid disease (1-5), in newborn children (6), and in both intact and parathyroidectomized rats (1, 7, 8). In these studies it has mostly been shown that dietary Pi alters either the fractional or the net amount of Pi reabsorbed by the renal tubule. Often these results have not been obtained at the same plasma concentration of Pi ([Pi]pl), since [Pi]pl depends on the ingested amount of Pi (9). Furthermore, most studies aimed at assessing an influence of dietary Pi on the renal tubule have been carried out either at endogenous [Pi]pl or at a level where maximum tubular reabsorption of Pi (TmPi) was supposed to take place (4, 10-13). Therefore such data cannot be interpreted as evidence for a modification of the tubular capacity to transport Pi. This requires in fact the demonstration of a change in the function which relates the tubular reabsorption of Pi to its plasma concentration and/or its filtered load. Thus any assessment of a diet-induced alteration in the tubular capacity necessitates the measurement of tubular Pi transport at least two [Pi]pl which

...continued
have to be comparable between the experimental groups.

There is only a single report in one child in whom Pi reabsorption was measured at both endogenous and acutely elevated plasma Pi before and after intestinal absorption had been reduced by aluminium hydroxide gel (6). The results suggested that the renal tubule might change its capacity to transport Pi in response to dietary Pi. A similar conclusion was drawn from experiments with fasted and fed dogs in which the filtered load of Pi had been varied by infusing sodium sulphate or changing the time interval after feeding (14). However the influence of fasting on the renal handling of Pi is a problem of its own and should be considered separately from the effect of dietary Pi.

Thus, as pointed out in two recent reviews (15, 16), no conclusive evidence has been presented so far showing that variations in dietary Pi modify the capacity of the renal epithelium to translocate Pi. This uncertainty obviously prevented the drawing of any conclusions as to a possible role of PTH in the renal response to changes in the dietary input of Pi.

In the present work, we have therefore reconsidered this problem by studying the tubular handling of Pi in three groups of both intact and thyroparathyroidectomized (TPTX) rats pair-fed diets containing low, normal, and high amounts of Pi. Clearance measurements were carried out over a wide range of [Pi]p in conscious animals. It was found that a change in Pi intake induces an adaptation whereby the renal tubule alters its capacity to transport Pi according to homeostatic requirements. Such an adaptation was observed in intact and TPTX rats operated both during and before the administration of the experimental diets. Further investigations indicated that it cannot be attributed to differences in plasma [Ca], ECVE, or urinary pH. A time-course study in TPTX rats showed that this diet-induced change in the tubular handling of Pi was detectable as soon as 3 days after switching the animals from a normal to low or high Pi diets.

METHODS

Preparation of the animals. Male Wistar rats weighing 150–170 g and raised on a commercial chow food (Altromin 1314, Alttragge, Lage [Lippe], W. Germany) containing 1.1 g/100 g Ca and 1.2 g/100 g phosphorus* with free access to tap water were used.

In a first series of experiments the rats were fasted overnight and then pair-fed for 10 days diets containing a constant amount of Ca (1.2 g/100 g) and different

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*The basic diets contain both organic and inorganic phosphorus. They were analyzed for P before incineration of the samples (see analytical methods). Thus dietary contents are given in grams of total phosphorus (P) per 100 grams of dry food. However, the change in P in the diets was obtained by adding various amounts of Pi only. Therefore, in the text, references to the types of food will be given in terms of low, medium, or high P, diets.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Food and Phosphorus Intake during the 10 Days before the Clearance Experiment</th>
</tr>
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<tbody>
<tr>
<td>Group</td>
<td>Dietary P (g/100 g)</td>
</tr>
<tr>
<td>---------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Intact rats</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
</tr>
<tr>
<td>B</td>
<td>1.2</td>
</tr>
<tr>
<td>C</td>
<td>1.8</td>
</tr>
<tr>
<td>TPTX rats, operated during experimental diet</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.2</td>
</tr>
<tr>
<td>B</td>
<td>1.2</td>
</tr>
<tr>
<td>C</td>
<td>1.8</td>
</tr>
<tr>
<td>TPTX rats, operated before experimental diet</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.2</td>
</tr>
<tr>
<td>E</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Values represent mean ± SE. n = number of animals from which data are presented in Tables II, III, and IV and Figs. 1 and 3. See Methods for exact composition of diets TPTX rats receiving diets A, B, and C were operated on the 8th day of experimental diet. TPTX rats receiving diets D and E were operated 5 days before onset of the experimental diet.

amounts of P; i.e. diet A, 0.2 g/100 g; diet B, 1.2 g/100 g; and diet C, 1.8 g/100 g. This was obtained by the addition of sodium phosphate and calcium gluconate to a basic diet (Altromin 1370 containing 0.12 g/100 g Ca and 0.22 g/100 g P dry wt). The sodium content was kept constant (2.45 g/100 g) by the addition of NaCl wherever necessary. All rats had free access to distilled water. Since the basic diet was poor in vitamin D, the rats were given an oral supplement of 25 IU (0.625 μg = 1,625 pmol) vitamin D, in 0.25 ml of vegetable oil three times weekly. The food intake was measured and the ingested Pi calculated (Table 1).

Intact rats were pair-fed for 10 days with diet A, B, or C. On the 9th day, i.e. 24 h before the clearance experiment, a subtotal cystectomy was made under light ether anesthesia to reduce the dead space of the urinary tractus.

Other rats, hereafter referred to as TPTX, were pair-fed diet A, B, or C for 10 days. Surgical thyroparathyroidectomy and subtotal cystectomy were done under ether anesthesia 44–48 h before the start of the clearance experiment. At the end of the clearance study the TPTX rats were returned to the usual lab chow (Altromin 1314) and allowed tap water ad libitum for five days. They were then fasted overnight and their [Ca]p was determined the next morning. Data are only presented for rats which displayed a [Ca]p below 1.88 mM (≈ 7.5 mg/100 ml) under these conditions and which were therefore considered to be fully TPTX.

In a second series of experiments, the thyroparathyroidectomy was done 5 days before starting the experimental diets. The effectiveness of the removal of the parathyroid glands was judged by measuring [Ca]p, (after fasting as described above) 4 days after the operation. Two diets were then used during 10 days for pair-feeding (Table 1), i.e. diet D, 0.2 g/100 g P, 1.2 g/100 g Ca; and diet E 1.8 g/100 g P, 2.7 g/100 g Ca. The additional amount
of gluconate provided in diet E was matched by an equivalent amount of glucono-lactone in diet D. The calcium content of diet E was set at 2.7 g/100 g to prevent too large a fall in [Ca] in this group of rats, TPTX for a longer period. These rats were cysteonymized 20-24 h before the clearance experiment.

These first two series of experiments prompted us to study whether the renal response to P1 intake could be evidenced in less than 10 days of experimental diet.

Therefore, in a third series of experiments, rats were pair-fed with diet A or C for 5 and 3 days. Thyroparathyroidecply had been done 5 days before starting the experimental diets.

**Clearance experiments.** In each series of experiments, rats of all dietary groups were studied the same day. To obviate any influence of the circadian rhythm of P1 excretion, the experiments were started at the same time each day (8.30 a.m.). The methodology of the clearance measurements in conscious rats has been described earlier (17).

Briefly, a first dose (priming) of inulin (80 mg/kg body wt) was infused in 0.15 M NaCl or P1 at various concentrations were then infused by means of an Ismatec micropump (Ismatec SA, Zurich, Switzerland) with a delivering rate of 3.9-4.0 ml/h. In another set of experiments a solution containing 0.2 g/100 ml inulin and 0.15 M NaCl or P1 at various concentrations were then infused at a rate of 20 ml/h to expand the extracellular volume. The pH of all solutions was adjusted to 7.4; for the P1 solutions, this was achieved by combining a solution of 16% NaHPO4 with one of 84% Na2HPO4. The osmolality was adjusted to 300-310 mosmol/liter by the addition of NaCl, where necessary.

In one type of clearance experiment measurements were made at two [P1]p. The rats were perfused for 120 min with 0.15 M NaCl for equilibration. Urine was collected afterwards for 45 min (period I), before and at the end of which two blood samples (a and b) were taken from a dorsal hind limb vein. The rats were then equilibrated for another 120 min with 100 mM P1 and urine (period II), and two blood samples (c and d) were obtained as described above. Subsequently, some rats of each group were anesthetized with ether and aortic blood was obtained anaerobically, for the determination of the ultrafiltrable fraction of P1, while the infusion continued.

The clearances of inulin (Cm) and P1 (Cr1) were calculated for both the basic clearance period I and the P1-loading period II by the standard formula. The blood concentrations used were the arithmetical mean of samples a and b for period I and of c and d for period II. The filtered load of phosphate (FLP1 = [P1]p × Cm), the absolute and fractional excretions of P1 (UVP1, UVP1/FLP1) and of sodium (UVNa, UVNa/FLNa) and the absolute and fractional tubular reabsorption of phosphate (TRP1 = FLP1 - UVP1, TRP1/FLP1) were calculated likewise. No correction was made for incomplete ultrafiltrability of plasma P1. Absolute rates of excretion and reabsorption were expressed per milliliter of glomerular filtrate (GF). In two separate experiments urine was collected anaerobically under xylol and urine pH was measured.

To describe more completely the function relating tubular reabsorption of P1 and [P1]p, measurements were also made under stepwise-increasing i.v. loads of P1, instead of only one load. In these experiments, the animals were "equilibrated" with 0.15 M NaCl for 90 min. A first urine collection period of 30 min (I) was made, at the end of which a blood sample (a) was taken. The rats were then infused with P1 at stepwise-increasing doses (45-min equilibrations followed by 30-min urine collection periods): 60 μmol P1/rat-h (period II), 120 μmol P1/rat-h (period III), and 180 μmol P1/rat-h (period IV). Blood samples (b, c, and d) were again taken immediately at the end of each urine collection period. The parameters of renal handling of P1, cited above were calculated for every period I to IV.

**Analytical methods.** Urinary volume (V) was determined by weighing. Inulin was determined in plasma and urine by the anthrone method (18). P1 was determined in plasma, in ultrafiltrate of plasma, in urine, and in the diets colorimetrically as phosphomolybdate after reduction with 10% ascorbic acid solution (19). Ca concentrations were measured in plasma and in the diets by atomic absorption spectroscopy (Perkin Elmer Corp., Norwalk, Conn., model 290 B) after diluting the samples with 0.5% LaCl3. Na concentrations in plasma and urine were determined by flame photometry (EEL flame photometer, Evans Electro-selenium Ltd., Halstead, Essex, England). The diets were analyzed after incineration of the samples at 550°C for 24 h and dissolution of the ash in 0.1 N HCl. The osmolality of solutions for infusion was measured with an Advanced Osmometer (model 3W, Advanced Instruments, Inc., Needham Heights, Mass.). The ultrafilterable fraction of P1 in plasma was measured in vitro at 37°C in an Amicon cell (model 12, Amicon Corp., Lexington, Mass.), fitted with an XM 50 Diaflo (Amicon) ultrafilter and operated at a gas pressure of 4 atmospheres (15% CO2 and 95% air).

**Statistical analysis.** The experimental results are expressed as mean values±SE. Significance of the differences between groups were evaluated by Student's t test. Differences between two clearance periods within the same group were evaluated by paired sample analysis.

**RESULTS**

**Intact rats.** Results of clearance experiments carried out at two [P1]p are presented in Table II. In period I, when the animals were infused with isotonic NaCl, [P1]p measured at the time of the clearance period (i.e., 120 and 165 min after starting the infusion) did not differ markedly among the three groups. There was about a 20% reduction in Cm in group C as compared with groups A or B; FLP1, however, was the same in groups fed both low and high P1, being somewhat lower in the latter than in the rats fed the medium diet. At these comparable FLP1, the absolute and the fractional excretions of P1 (UVP1/GF), UVP1/FLP1 varied very markedly according to the prior dietary intake of P1, being about 1,000 times lower on the low P1 diet (A) than on the high P1 diet (C), with the values of the medium diet (B) lying in between. [Ca]p tended to be higher in animals fed a low P1 diet than in rats on larger P1 intake.

In period II, when the animals received an acute i.v. load of P1, [P1]p was not significantly different among the three groups. P1 infusion did not modify Cm. In spite of a larger FLP1 in group A than in group C (P <
TABLE II

Diet-Induced Change in the Renal Handling of Phosphate in Intact Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary P</th>
<th>n</th>
<th>Body wt</th>
<th>Period</th>
<th>Cis</th>
<th>V</th>
<th>[Pi]pi</th>
<th>FLPi</th>
<th>UVPi</th>
<th>UVPi/FLPi</th>
<th>TRPi</th>
<th>UVwa</th>
<th>[Ca]pi</th>
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<tbody>
<tr>
<td>A</td>
<td>0.2</td>
<td>7</td>
<td>172</td>
<td>I</td>
<td>1.31</td>
<td>46</td>
<td>1.91*</td>
<td>2.51</td>
<td>0.0008</td>
<td>0.047**</td>
<td>1.88</td>
<td>6.74</td>
<td>2.74*</td>
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<tr>
<td></td>
<td>±0.00</td>
<td>±7</td>
<td>±0.11</td>
<td></td>
<td>±0.18</td>
<td></td>
<td>±0.002</td>
<td>±0.01</td>
<td>±0.12</td>
<td>±0.43</td>
<td>±0.11</td>
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<tr>
<td></td>
<td>II</td>
<td>1.45</td>
<td>67</td>
<td>5.56</td>
<td>8.01</td>
<td>3.62*</td>
<td>565.12</td>
<td>1.95**</td>
<td>9.92</td>
<td>1.61</td>
<td></td>
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<tr>
<td></td>
<td>±0.08</td>
<td>±0</td>
<td>±0.17</td>
<td></td>
<td>±0.11</td>
<td></td>
<td>±2.1</td>
<td>±0.10</td>
<td>±0.72</td>
<td>±0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.2</td>
<td>6</td>
<td>196</td>
<td>I</td>
<td>1.34</td>
<td>52</td>
<td>2.53</td>
<td>3.41</td>
<td>0.495</td>
<td>19.55</td>
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<tr>
<td></td>
<td>±0.15</td>
<td>±4</td>
<td>±0.05</td>
<td></td>
<td>±0.42</td>
<td></td>
<td>±6.0</td>
<td>±0.10</td>
<td>±0.94</td>
<td>±0.02</td>
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<tr>
<td></td>
<td>II</td>
<td>1.33</td>
<td>72</td>
<td>5.82</td>
<td>7.68</td>
<td>4.60</td>
<td>79.0</td>
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<td>11.05</td>
<td>1.81</td>
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<tr>
<td></td>
<td>±0.10</td>
<td>±8</td>
<td>±0.18</td>
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<td>±0.42</td>
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<td>±2.5</td>
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<td>±0.50</td>
<td>±0.09</td>
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<tr>
<td>C</td>
<td>1.8</td>
<td>6</td>
<td>183</td>
<td>I</td>
<td>1.06</td>
<td>56</td>
<td>2.27*</td>
<td>2.39**</td>
<td>1.03*</td>
<td>45.2*</td>
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<tr>
<td></td>
<td>±0.05</td>
<td>±5</td>
<td>±0.10</td>
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<td>±3.5</td>
<td>±0.07</td>
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<td>±0.06</td>
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<tr>
<td></td>
<td>II</td>
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<td>75</td>
<td>5.34</td>
<td>6.15*</td>
<td>5.16</td>
<td>96.71</td>
<td>0.18*</td>
<td>10.43</td>
<td>1.88</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>±0.05</td>
<td>±5</td>
<td>±0.14</td>
<td></td>
<td>±0.20</td>
<td></td>
<td>±4.5</td>
<td>±0.78</td>
<td>±0.03</td>
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</tr>
</tbody>
</table>

Animals were fed diet A, B, or C for 10 days (see Methods). The infused solutions (4 ml/h) were in period I 0.15 M NaCl and in period II 0.10 M Na3PO4/NaHPO4 +0.03 M NaCl. Cis, clearance of insulin; V, urinary volume; [Pi]pi, phosphate; FLPi, filtered load of phosphate; UVPi, urinary excretion of phosphate; UVPi/FLPi, X 100, fractional excretion of phosphate; TRPi, tubular reabsorption of phosphate; [Ca]pi, calcemia. Values represent mean±SE, n, number of animals.

* P < 0.05
† P < 0.01.
§ P < 0.001 as compared with the corresponding value of group B.

0.01), UVPi/GF and UVPi/FLPi were lower in group A than in groups B and C (P < 0.001), thus being again related to dietary intake. It is important to note that rats fed the low-Pi diet responded differently to the same intravenous load of Pi from animals fed higher Pi diets. Their reabsorption of Pi (TRPi/GF) was maintained at a similar level, whereas it fell sharply in the rats fed the higher Pi diet. This difference in response led to a significantly lower TRPi/GF in group C as compared to group A (P < 0.001). The acute Pi infusion led to a diminution of [Ca]pi in all three groups, the fall in [Ca]pi being greater in group A than in groups B and C. This resulted in a significantly lower calcemia in the group A when compared with group C (P < 0.001).

The ultrafilterability of Pi determined at the end of one clearance experiment was the same in all three dietary groups, i.e. group A, 94.6±2.3% (n = 3); group B, 98.1% and 95.7% (n = 2); group C, 93.3±1.3% (n = 4), respectively. These results correspond to those previously reported for the rat at [Pi]pi ranging from 1 to 14 mM (20).

Two experiments to determine urinary pH were conducted with animals fed diets A and C for 10 days. In period I, it was higher in the urine of rats on the low Pi diet than in those on the high Pi diet: 6.70±0.13 (n = 12) compared with 5.89±0.18 (n = 10), P < 0.01. In period II, when Pi was infused at pH 7.4, this difference was not observed: group A, 6.84±0.10; group C, 6.86±0.18.

A more complete description of the relationship between [Pi]pi and tubular reabsorption of Pi is presented.

Figure 1 Fractional reabsorption of Pi determined under acute i.v. sodium chloride and stepwise-increasing sodium phosphate infusions (4 ml/h, as described in methods) in 24 intact rats pair-fed diets containing either 0.2 g/100 g P (n = 12; ○) or 1.8 g/100 g P (n = 12; ▲). Values represent mean±SE. Data obtained between each millimolar unit of plasma Pi concentration were pooled. *P < 0.001.

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in Fig. 1. It contains the results from the clearance experiments of groups A and C described above and results from experiments carried out with stepwise-increasing i.v. loads of \([P_i]\). The fractional reabsorption of \([P_i]\) (TRP/FLPi) was always higher in rats fed the low \([P_i]\) (A) than in those fed the high \([P_i]\) diet (C) for any given \([P_i]\) over a wide range.

Since ECVE has been shown to decrease the tubular reabsorption of \([P_i]\) in intact rats (21), it was necessary to investigate whether our results were due to a change in volume expansion. UVPi/GF was not significantly different between rats fed the low or the high \([P_i]\) diet as compared to the control group during period I and II (Table II). However, this does not allow us to exclude a critical role of ECVE because of the larger variation in UVPi/GF than in UVPi/GF. On the other hand, even a significant difference in the renal handling of Na would not mean that the effect of the diets on the tubular transport of \([P_i]\) resulted from a primary change in sodium transport consecutive to an alteration in ECVE. Indeed, the diet-induced change in the tubular handling of \([P_i]\) could well affect secondarily the transport of a cation, e.g. sodium. Therefore, a conclusive way to assess whether uneven ECVE accounts for this change in \([P_i]\) handling is to evaluate whether the difference persists under marked ECVE. This was achieved in intact rats by infusing 0.15 M saline at a rate of 20 ml/h. It lead to a 10% increase in body weight and promoted a fractional excretion of Na of about 10% in rats fed both low and high \([P_i]\) diets (Fig. 2). In presence of such a marked ECVE, comparable to that achieved in Frick’s study (21), and in the presence of a similar [P]i, fractional excretion of \([P_i]\) (UVPi/FLPi × 100) was 200 times greater in rats on a high \([P_i]\) diet: low \([P_i]\) diet, 0.10±0.001 (n = 5); high \([P_i]\) diet, 23.9±5.4 (n = 6), P < 0.001 (Fig. 2). CNa was also not significantly different: low \([P_i]\) diet, 2.02±0.06; high \([P_i]\) diet, 1.75±0.21 ml/min. Thus the diet-induced difference in the tubular handling of \([P_i]\) described above was not abolished in the presence of a conspicuous ECVE.

**TPTX rats.** To assess whether the changes in \([P_i]\) handling are due to a change in PTH secretion, clearance experiments were carried out at two [P]i in TPTX animals operated 44-48 h previously. The results are presented in Table III. In the unloaded state (period I) TPTX rats fed a low-\([P_i]\) diet had a [P]i significantly lower than those fed control or high-\([P_i]\) diets (P < 0.001). Since CNa was about the same in all three groups, FLPi was also lower in group A as compared to groups B and C (P < 0.001). Again UVPi and UVPi/FLPi were related to the previous dietary intake of \([P_i]\). Thus, although FLPi was somewhat higher after 1.2 g/100 g P (group B) than in the other two groups, UVPi and UVPi/FLPi of this medium group was between groups A and C. [Ca]Pi was highest in the rats fed the low \([P_i]\) diet (A).

Under intravenous phosphate loading (period II), the TPTX rats had a higher [P]i in group A than in group C (P < 0.001), but FLPi were comparable. Nev-

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TABLE III

Diet-Induced Change in the Renal Handling of Phosphate in TPTX Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary P</th>
<th>n</th>
<th>Body wt</th>
<th>Period</th>
<th>Ca&lt;sub&gt;x&lt;/sub&gt;</th>
<th>V</th>
<th>[P]&lt;sub&gt;i&lt;/sub&gt;/V</th>
<th>FLP&lt;sub&gt;i&lt;/sub&gt;</th>
<th>UV&lt;sub&gt;P&lt;/sub&gt;</th>
<th>UV&lt;sub&gt;P&lt;/sub&gt;/FLP&lt;sub&gt;i&lt;/sub&gt;</th>
<th>TRP&lt;sub&gt;i&lt;/sub&gt;</th>
<th>UV&lt;sub&gt;Na&lt;/sub&gt;</th>
<th>[Ca]&lt;sub&gt;i&lt;/sub&gt;/Pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.2</td>
<td>6</td>
<td>158</td>
<td>I</td>
<td>1.24±0.04</td>
<td>47</td>
<td>1.57±0.02</td>
<td>1.94±0.08</td>
<td>0.0018±0.0006</td>
<td>0.11±0.037</td>
<td>1.57±0.03</td>
<td>4.95±0.38</td>
<td>2.42±0.06</td>
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<td>±4</td>
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<td></td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.06</td>
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<tr>
<td>II</td>
<td>1.32±0.14</td>
<td>27</td>
<td>6.59</td>
<td>I</td>
<td>1.42±0.09</td>
<td>49</td>
<td>3.15±0.15</td>
<td>4.50±0.46</td>
<td>0.081±0.039</td>
<td>2.45±1.1</td>
<td>3.07±0.11</td>
<td>5.11±0.57</td>
<td>1.52±0.06</td>
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<td></td>
<td></td>
<td>±9</td>
<td></td>
<td></td>
<td>±0.15</td>
<td>±1.1</td>
<td>±0.06</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>1.8</td>
<td>5</td>
<td>173</td>
<td>I</td>
<td>1.34±0.13</td>
<td>34</td>
<td>2.82±0.14</td>
<td>3.76±0.36</td>
<td>0.399±0.083</td>
<td>13.76±2.2</td>
<td>2.42±0.06</td>
<td>5.13±0.69</td>
<td>1.71±0.06</td>
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<td></td>
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<td>±6</td>
<td></td>
<td></td>
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<td>±2.2</td>
<td>±0.06</td>
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<tr>
<td>II</td>
<td>1.49±0.05</td>
<td>57</td>
<td>5.81±0</td>
<td>I</td>
<td>1.49±0.10</td>
<td>57</td>
<td>5.81±0.18</td>
<td>8.67±0.38</td>
<td>4.48±0.13</td>
<td>7.80±2.75</td>
<td>1.33±0.18</td>
<td>7.89±0.79</td>
<td>1.12±0.16</td>
</tr>
</tbody>
</table>

Thyroparathyroidecetomy was performed 8 days after starting the experimental diets, i.e., 2 days before the clearance experiment. See legend to Table II for further explanations.

ertheless, UV<sub>P</sub> and UV<sub>P</sub>/FLP<sub>i</sub> were lower in group A than in group C (P < 0.01 for UV<sub>P</sub>/FLP<sub>i</sub>). As observed in the intact rats, TPTX animals fed a low Pi diet responded to the same intravenous load of Pi differently from animals on higher Pi intake. TRP<sub>i</sub>/GF slightly increased in group A, whereas it markedly decreased in the rats fed higher Pi diets. This different response led to a significantly lower TRP<sub>i</sub>/GF in group C as compared to group A (P ≤ 0.001).

Results from these clearance experiments and of those performed with stepwise-increasing i.v. loads of Pi are shown in Fig. 3. TRP<sub>i</sub>/FLP<sub>i</sub> measured at [P]<sub>i</sub>/V ranging from 1 to 7.5 mM in rats was always higher for a given [P]<sub>i</sub>/V in the animals fed the low-Pi diet (A) than in those receiving the high-Pi diet (C).

As in the intact rats, there was no significant difference in UV<sub>Na</sub>/GF between rats fed the low or high Pi diet as compared to the control group during both periods I and II.

The calcemia per se has been implicated in the regulation of Pi excretion. It is noteworthy in this respect that in the TPTX animals the difference in the tubular reabsorption illustrated in Fig. 3 was not accompanied by a difference in [Ca]<sub>i</sub>/Pi for a given [P]<sub>i</sub>/V (Fig. 4).

Very recent studies indicate that the disappearance of PTH from the circulation is extremely rapid in the rat. Indeed, its half-life is very short, 99% of an injected dose leaving the circulation in 60 min (22). It follows that no circulating PTH was present at the time of the clearance studies carried out 48 h after the removal of the parathyroid glands. But it remains possible that PTH could have played a role in the induction of the adaptation phenomenon between the onset of the experimental diet and the thyroparathyroidectomy. Therefore rats were also TPTX 5 days before being fed low- or high-Pi diets for 10 days. The effectiveness of parathyroidectomy was assessed 4 days after surgery: [Ca]<sub>i</sub>/Pi was 1.53±0.1 (n = 4) and 1.36±0.11 mM (n = 5) in the group to be fed the low- and the high-Pi diet, respectively. The results of the clearance experiment are shown in Table IV. In the unloaded state (period I), at comparable [P]<sub>i</sub>/V and FLP<sub>i</sub>, UV<sub>P</sub>/FLP<sub>i</sub> was significantly lower in the animals fed the low-Pi diet (D) than in those fed the high-Pi diet (E). Under intravenous loading with P<sub>i</sub> (period II), [P]<sub>i</sub>/V was not significantly different between the two dietary groups. Although FLP<sub>i</sub> was higher in the group on low P<sub>i</sub> intake (D), its UV<sub>P</sub>/
Thyroparathyroidectomy was performed 5 days before starting the experimental diets, i.e., 15 days before the clearance experiment. Values represent mean±SE. n = number of animals. See legend to Table II for further explanation.

* P < 0.05. † P < 0.001 as compared with the corresponding value of group E.

FLP1 was significantly lower than in the group on high P1 intake (E). No significant differences in UVNa/GF were found. As observed in intact rats (Table II) and in rats TPTX while under the experimental diet (Table III), the response to acute P1 loading was also different according to the prior dietary intake of P1. It led to a significantly lower TRP1/GF in group E as compared to group D. These results indicate that PTH or other hormones of the thyroparathyroid glands are not essential for the induction of the tubular adaptation to a change in P1 intake.

All the experiments presented so far have been done in rats fed the experimental diets for 10 days. To study whether the renal response to the dietary P1 intake could be detected earlier, clearances were done after shorter times in rats TPTX both before and after starting the experimental diet. In this latter group the renal study was carried out 3 and 5 days after switching the diet from 1.2 g/100 g P to 0.2 g/100 g P (diet A) or to 1.8 g/100 g P (diet C). Food intake was 10.8±0.2 and 10.2±0.4 g/rat·day in group A and C, respectively, during the experimental period of 3 days. It was 16.9±0.2 and 15.5±0.3 g/rat·day during the experimental period of 5 days. The fractional reabsorptions of P1 measured under isotonic NaCl infusion are depicted in Fig. 5. In this figure the data from Table III, obtained after a 10 days’ dietary period, are also plotted. After 3 days, there already was a significant difference (P < 0.01) in the fractional reabsorption of P1 between low and high P1-fed animals. This was measured at similar [P1]p (low 3.19±0.26, n = 5; high 3.38±0.41 mM, n = 4), C4 (1.31±0.06 and 1.29±0.09 ml/min), and FLP1 (4.18±0.43 and 4.42±0.75 μmol/min). In group A, prolonging the low-P1 diet led to a decrease in [P1]p (day 3, 3.19±0.25; day 5, 2.74±0.05; day 10, 1.57±0.02 mM). In group C the fractional reabsorption of P1 decreased with time; [P1]p tended to increase from day 3 (3.23±0.35 mM) to day 5 (4.09±0.15 mM) but returned after 10 days to values observed under the 1.2 g/100 g P diet (compare also Table III). This return of [P1]p to its initial value might be related to the further fall in the tubular reabsorption occurring between days 5 and 10. The effect of a dietary period of 3 days was also studied in a series of rats TPTX 5 days before starting the experimental diets. 4 days after surgery, [Ca1]p had fallen to 1.51±0.06 (n = 8) and 1.47±0.05 mM (n = 6) in the group to be fed the low (D) and high (E) P1.

**Figure 5** Fractional reabsorption of P1 in TPTX rats pair-fed diets containing either 0.2 g/100 g P (○) or 1.8 g/100 g P (△) for 3, 5, or 10 days. The data obtained with a 1.2 g/100 g P diet (×) are also presented. Values at 10 days correspond to those given in Table III. Thyroparathyroidectomy was done 2 days before the clearance experiment.

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**Table IV**

**Diet-Induced Change in the Renal Handling of Phosphate in Rats TPTX before the Onset of the Experimental Diets**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dietary P</th>
<th>n</th>
<th>Body wt</th>
<th>Period</th>
<th>Ci0</th>
<th>[P1]p</th>
<th>FLP1</th>
<th>UVPi/FLP1</th>
<th>TRP1</th>
<th>UVPi</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td></td>
<td></td>
<td></td>
<td>ml/min</td>
<td>mM</td>
<td>μmol/min</td>
<td>×100</td>
<td>μmol/ml GF</td>
<td>μmol/ml GF</td>
</tr>
<tr>
<td>D</td>
<td>0.2</td>
<td>4</td>
<td>211</td>
<td>I</td>
<td>1.29</td>
<td>2.48</td>
<td>3.11</td>
<td>0.61</td>
<td>2.48</td>
<td>6.99</td>
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<td></td>
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<td></td>
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<td>±0.23</td>
<td>±0.05</td>
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<td>±1.57</td>
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<tr>
<td>II</td>
<td>1.17</td>
<td>6.59</td>
<td>7.66*</td>
<td>65.3*</td>
<td>2.27†</td>
<td>11.82</td>
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<tr>
<td></td>
<td>±0.04</td>
<td>±0.38</td>
<td>±0.42</td>
<td>±1.7</td>
<td>±0.12</td>
<td>±0.82</td>
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<tr>
<td>E</td>
<td>1.8</td>
<td>5</td>
<td>199</td>
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<td>±1.7</td>
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<tr>
<td>II</td>
<td>0.97</td>
<td>6.61</td>
<td>6.40</td>
<td>77.8</td>
<td>1.49</td>
<td>11.72</td>
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<tr>
<td></td>
<td>±0.04</td>
<td>±0.69</td>
<td>±0.26</td>
<td>±4.3</td>
<td>±0.30</td>
<td>±0.45</td>
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</table>
diets. During the following dietary period of 3 days, their food intake was 8.8±0.5 and 9.0±0.4 g/rat·day. In the clearance experiment, [Pi]tr was lower after Pi deprivation (low, 2.77±0.12, n = 8; high, 3.41±0.06 mM, n = 6; P < 0.01). But because Cm was slightly higher in this group (low, 1.51±0.07; high, 1.29±0.09 ml/min, NS), both groups displayed the same FLPi (low, 4.19±0.24; high, 4.40±0.28 μmol/min). In this condition, as in the preceding series, a marked difference in the fractional reabsorption of Pi was observed between the two dietary groups. The animals fed the low-Pi diet reabsorbed virtually all Pi filtered (99.3±0.3%), whereas the rats on high Pi intake reabsorbed only 95.2±1.0% (P < 0.01). Thus it appears that the adaptive response of the tubule to dietary Pi is detectable after 3 days in rats both during and before the start of the experimental diets.

DISCUSSION

The present study demonstrates that the renal tubule responds to variations in Pi intake by modulating its capacity to reabsorb Pi. Indeed, the diet-induced change in the fractional reabsorption of Pi exceeds that expected merely from variations in the filtered load of Pi. The capacity of the tubule to reabsorb Pi varies according to homeostatic requirements, being greater under a low- than under a high-Pi diet, indicating an adaptation phenomenon. Removal of the thyroparathyroid glands, done either before or after the onset of the dietary treatment, does not abolish the tubular adaptive response, which can be observed as early as 3 days after switching rats to low- or high-Pi diets. However, it diminishes the ability of the renal tubule to adapt to a high Pi intake. Indeed, the capacity of the tubule to reabsorb Pi remains greater in TPTX rats fed high-Pi diets than in intact rats (Tables II and III). This might be responsible for their higher [Pi]tr. The response to an acute infusion of Pi differs markedly according to the preceding Pi intake in both intact and TPTX rats. In the intact animals fed a low Pi diet, the net tubular reabsorption did not change, as shown in Table II. This result would be consistent with the classical concept of a maximum and constant transfer of Pi (TmPi) across the tubular epithelium over a wide range of plasma phosphate concentrations. In intact rats fed a high Pi diet the acute elevation of plasma Pi led to a sharp decrease of the tubular reabsorption. Therefore under these conditions no apparent TmPi could be observed, confirming previous observations in rats (23, 24). Thus the demonstration of a TmPi seems to depend, at least in the rat, upon the amount of phosphate previously ingested. This of course casts some doubt on the actual physiological significance of the Tm value for inorganic phosphate in the rat.

The fall of TRPi/GF to values not significantly different from 0 in some rats fed a high-Pi diet (Table II) suggests strongly the existence of a secretory flux of Pi in this condition. Evidence for such a tubular secretion has recently been obtained in our laboratory by micropuncture and microperfusion techniques (24).

These findings show clearly that factors other than PTH are involved in the control of renal Pi transport. Several as yet contradictory results of studies recently reviewed (15, 16, 25) have pointed to the possible role of the calcemia per se in the renal handling of Pi. The higher plasma calcium concentration observed in TPTX rats fed the low Pi diet could be directly related to the higher fractional reabsorption displayed by these animals under NaCl infusion. However, under Pi infusion the difference in the tubular capacity to transport Pi was observed with no difference in [Ca] in for any given [Pi]tr. This does not exclude the possibility that the diet-induced chronic change in calcemia might have played a role in the alteration of the tubular handling of Pi.

ECVE has been shown to decrease the tubular reabsorption of Pi in intact rats (21, 23, 26, 27). In TPTX rats, however, the increase in phosphate excretion in response to ECVE has been reported to be blunted (21, 27). In our conditions, the differences in the tubular reabsorption of Pi induced by the dietary intake of Pi was maintained under marked ECVE in intact rats. Thus a change in ECVE does not seem to be implicated in the tubular response to variations in Pi intake.

Phosphate-depleted dogs have been reported to excrete an increased amount of bicarbonate (28). This might account for the higher urinary pH observed at endogenous [Pi]tr in our rats on low Pi diet. This finding, however, cannot explain the greater tubular reabsorption observed in the rats fed the low Pi diet: indeed, alkalinization of urine has been shown to decrease the tubular reabsorption of Pi in dogs and in man (29–31). Furthermore, the urinary pH was the same in both groups during the acute load with Pi, while the difference in the handling of Pi still occurred.

Our data show that the diet-induced modulation of the renal tubular capacity to transport Pi is in part independent of PTH, of [Ca] in at the time of the clearance measurement, of ECVE, and of urinary pH. It is possible that vitamin D3 metabolites could be among the factors involved in the tubular adaptation. Indeed some of the active metabolites of vitamin D3 have been shown to enhance the renal reabsorption of Pi (32, 33). Furthermore, Pi intake alters the production of 1,25-dihydroxycholecalciferol (34). Very recently it has been reported that vitamin D-repleted TPTX rats on a very low Pi diet (0.05 g/100 g) excreted virtually no Pi at filtered loads up to 5 μmol/min, whereas vitamin D-free TPTX rats excreted significant quantities, especially at
filtered loads above 2 μmol/min (35). However, in an abstract, vitamin D-free TPTX rats on a low Pi diet were reported to reabsorb practically all Pi filtered (36). Thus the role of vitamin D, if any, is still not clear.

In conclusion, the present work shows that the renal tubule can adapt its capacity to transport Pi in response to dietary Pi. It indicates the existence of an as yet unknown regulatory factor involved in the urinary excretion of Pi. The exact mechanism underlying this renal tubular adaptation phenomenon as well as its localization along the nephron remain to be elucidated.

ACKNOWLEDGMENTS

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