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Research Article

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 active 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]P1). 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]P1, 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.

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 (P_i) was investigated in intact and thyroparathyroidectomized (TPTX) rats pair-fed diets containing low, normal, and high amounts of P_i for periods up to 10 days. Clearances were measured before and during acute i.v. infusions with P_i in conscious animals. Thus tubular reabsorption of phosphate (TRP_i) could be assessed over a wide range of plasma phosphate concentrations ($[P_i]_{Pi}$). It was found that the renal tubule could adapt its capacity to transport P_i according to the dietary P_i: TRP_i was always higher, for a given $[P_i]_{Pi}$, in the animals fed low than in those fed higher P_i 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 P_i was detectable as early as 3 days after switching the animals from a normal to low- or high-P_i 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 (P_i)[1] 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 P_i 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 P_i alters either the fractional or the net amount of P_i reabsorbed by the renal tubule. Often these results have not been obtained at the same plasma concentration of P_i ($[P_i]_{Pi}$), since $[P_i]_{Pi}$ depends on the ingested amount of P_i (9). Furthermore, most studies aimed at assessing an influence of dietary P_i on the renal tubule have been carried out either at endogenous $[P_i]_{Pi}$ or at a level where maximum tubular reabsorption of P_i (TmP_i) 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 P_i. This requires in fact the demonstration of a change in the function which relates the tubular reabsorption of P_i 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 P_i transport at at least two $[P_i]_{Pi}$ which

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[1] *Abbreviations used in this paper:* C_{In}, clearance of inulin; C_{Pi}, clearance of inorganic phosphate; ECVE, extracellular volume expansion; FLP_i, filtered load of phosphate; FLNa, filtered load of sodium; GF, glomerular filtrate; P_i, inorganic phosphate; $[P_i]_{Pi}$, plasma phosphate concentration; PTH, parathyroid hormone; TmP_i, maximum tubular reabsorption of P_i; TPTX, thyroparathyroidectomized; TRP_i, tubular reabsorption of phosphate; TRP_i/FLP_i, fractional tubular reabsorption of phosphate; UV, absolute excretion; UVP_i/FLP_i, fractional excretion of P_i; V, urinary volume.

have to be comparable between the experimental groups.

There is only a single report in one child in whom P_i reabsorption was measured at both endogenous and acutely elevated plasma P_i 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 P_i in response to dietary P_i. A similar conclusion was drawn from experiments with fasted and fed dogs in which the filtered load of P_i 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 P_i is a problem of its own and should be considered separately from the effect of dietary P_i.

Thus, as pointed out in two recent reviews (15, 16), no conclusive evidence has been presented so far showing that variations in dietary P_i modify the capacity of the renal epithelium to translocate P_i. 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 P_i.

In the present work, we have therefore reconsidered this problem by studying the tubular handling of P_i in three groups of both intact and thyroparathyroidectomized (TPTX) rats pair-fed diets containing low, normal, and high amounts of P_i. Clearance measurements were carried out over a wide range of $[P_i]_{P_i}$ in conscious animals. It was found that a change in P_i intake induces an adaptation whereby the renal tubule alters its capacity to transport P_i 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 P_i was detectable as soon as 3 days after switching the animals from a normal to low or high P_i diets.

METHODS

Preparation of the animals. Male Wistar rats weighing 150–170 g and raised on a commercial chow food (Altromin 1314, Altragge, Lage [Lippe], W. Germany) containing 1.1 g/100 g Ca and 1.2 g/100 g phosphorus[a] 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 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 C 1730 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 where 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_3 in 0.25 ml of vegetable oil three times weekly. The food intake was measured and the ingested P_i calculated (Table I).

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 $[Ca]_{P_i}$ was determined the next morning. Data are only presented for rats which displayed a $[Ca]_{P_i}$ 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 $[Ca]_{P_i}$ (after fasting as described above) 4 days after the operation. Two diets were then used during 10 days for pair-feeding (Table I), 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

TABLE I

Food and Phosphorus Intake during the 10 Days before the Clearance Experiment

Group	Dietary P	n	Mean daily food intake	Mean daily intake of P
	g/100 g			
Intact rats				
A	0.2	12	17.2±0.5	34±1
B	1.2	6	17.7±0.4	212±5
C	1.8	12	16.4±0.7	295±13
TPTX rats, operated during experimental diet				
A	0.2	16	14.1±0.6	28±1
B	1.2	4	14.8±0.7	178±9
C	1.8	12	14.3±0.8	257±14
TPTX rats, operated before experimental diet				
D	0.2	4	16.7±0.9	33.5±2
E	1.8	5	18.7±1.2	337±22

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.

[a] The basic diets contain both organic and inorganic phosphorus. They were analyzed for P_i after 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 P_i only. Therefore, in the text, references to the types of food will be given in terms of low, medium, or high P_i diets.

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 $[Ca]_{PI}$ in this group of rats, TPTX for a longer period. These rats were cystectomized 20–24 h before the clearance experiment.

These first two series of experiments prompted us to study whether the renal response to P_I 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. Thyroparathyroidectomy was performed 44–48 h before the clearance experiment, as in the first series of experiments.

Finally, in a fourth series of experiments, rats were pair-fed diets D or E for 3 days. But, as in the second series of experiments, thyroparathyroidectomy 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 P_I 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), dissolved in 0.15 M NaCl, was injected i.v. in a volume of 2.5 ml/kg body wt. Solutions containing 1 g/100 ml of inulin and either 0.15 M NaCl or P_I 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 was 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 P_I solutions, this was achieved by combining a solution of 16% NaH_2PO_4 with one of 84% Na_2HPO_4. 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 $[P_I]_{PI}$. 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 P_I 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 P_I, while the infusion continued.

The clearances of inulin (C_{In}) and P_I (C_{PI}) were calculated for both the basic clearance period I and the P_I-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 ($FLP_I = [P_I]_{PI} \times C_{In}$), the absolute and fractional excretions of P_I (UVP_I, UVP_I/FLP_I) and of sodium (UVNa, UVNa/FLNa) and the absolute and fractional tubular reabsorption of phosphate ($TRP_I = FLP_I - UVP_I$, TRP_I/FLP_I) were calculated likewise. No correction was made for incomplete ultrafiltrability of plasma P_I. 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 P_I and $[P_I]_{PI}$, measurements were also made under stepwise-increasing i.v. loads of P_I 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 P_I at stepwise-increasing doses (45-min equilibrations followed by 30-min urine collection periods): 60 μmol P_I/rat·h (period II), 120 μmol P_I/rat·h (period III), and 180 μmol P_I/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 P_I 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). P_I 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% $LaCl_3$. Na concentrations in plasma and urine were determined by flame photometry (EEL flame photometer, Evans Electroselenium Ltd., Halstead, Essex, England). The diets were analyzed after incineration of the samples at 650°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 P_I 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 (1% CO_2 and 99% 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 $[P_I]_{PI}$ are presented in Table II. In period I, when the animals were infused with isotonic NaCl, $[P_I]_{PI}$ 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 C_{In} in group C as compared with groups A or B; FLP_I, however, was the same in groups fed both low and high P_I, being somewhat lower in the latter than in the rats fed the medium diet. At these comparable FLP_I, the absolute and the fractional excretions of P_I (UVP_I/GF), UVP_I/FLP_I) varied very markedly according to the prior dietary intake of P_I, being about 1,000 times lower on the low P_I diet (A) than on the high P_I diet (C), with the values of the medium diet (B) lying in between. $[Ca]_{PI}$ tended to be higher in animals fed a low P_I diet than in rats on larger P_I intake.

In period II, when the animals received an acute i.v. load of P_I, $[P_I]_{PI}$ was not significantly different among the three groups. P_I infusion did not modify C_{In}. In spite of a larger FLP_I in group A than in group C ($P <$

TABLE II

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

Group	Dietary P	n	Body wt	Period	C_{In}	V	$[P_i]_{PI}$	FLP_i	UVP_i	UVP_i/FLP_i	TRP_i	UV_{Na}	$[Ca]_{PI}$
	g/100 g		*g*		*ml/min*	*μl/min*	*mM*	*μmol/min*	*μmol/ml GF*	*×100*	*μmol/ml GF*	*μmol/ml GF*	*mM*
A	0.2	7	172 ±4	I	1.31 ±0.06	46 ±7	1.91* ±0.11	2.51 ±0.18	0.0008§ ±0.0002	0.047§ ±0.010	1.88 ±0.12	6.74 ±0.43	2.74* ±0.11
				II	1.45 ±0.08	67 ±6	5.56 ±0.17	8.01 ±0.41	3.62‡ ±0.11	65.1‡ ±2.1	1.95‡ ±0.16	9.92 ±0.72	1.61 ±0.04
B	1.2	6	196 ±2	I	1.34 ±0.15	52 ±4	2.53 ±0.05	3.41 ±0.42	0.495 ±0.10	19.55 ±4.0	2.04 ±0.10	8.02 ±0.94	2.41 ±0.02
				II	1.33 ±0.10	72 ±8	5.82 ±0.18	7.68 ±0.42	4 60 ±0.25	79.0 ±3.0	1.21 ±0.20	11.05 ±0.50	1.81 ±0.09
C	1.8	6	183 ±5	I	1.06 ±0.05	56 ±5	2.27* ±0.10	2.39* ±0.13	1.03§ ±0.11	45.2§ ±3.5	1.23§ ±0.07	9.39 ±0.53	2.32 ±0.06
				II	1.16 ±0.05	75 ±5	5.34 ±0.14	6.15‡ ±0.20	5.16 ±0.24	96.7‡ ±4.5	0.18‡ ±0.24	10.43 ±0.78	1.88 ±0.03

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 $Na_2PO_4/NaHPO_4$ +0.03 M NaCl. C_{In}, clearance of inulin; V, urinary volume; $[P_i]PI$., phosphatemia; FLP_i, filtered load of phosphate; UVP_i, urinary excretion of phosphate, $UVP_i/FLP_i \times 100$, fractional excretion of phosphate; TRP_i, 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), UVP_i/GF and UVP_i/FLP_i 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-P_i diet responded differently to the same intravenous load of P_i from animals fed higher P_i diets. Their reabsorption of P_i (TRP_i/GF) was maintained at a similar level, whereas it fell sharply in the rats fed the higher P_i diet. This difference in response led to a significantly lower TRP_i/GF in group C as compared to group A ($P < 0.001$). The acute P_i 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 P_i 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 $[P_i]_{PI}$ 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 P_i diet than in those on the high P_i diet: 6.70±0.13 ($n = 12$) compared with 5.89±0.18 ($n = 10$), $P < 0.01$. In period II, when P_i 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 $[P_i]_{PI}$ and tubular reabsorption of P_i is presented

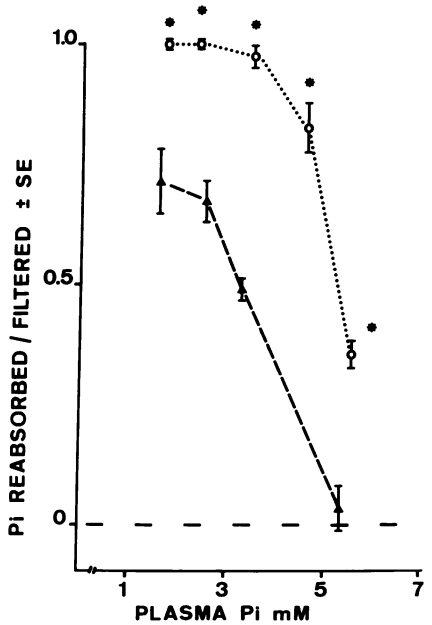

FIGURE 1 Fractional reabsorption of P_i determined under acute i.v. sodium chloride and stepwise-increasing sodium phosphate infusions (4 ml/h, as described in methods) in 24 intact rats pair-fed diets containing either 0.2 g/100 g P ($n = 12$; ○) or 1.8 g/100 g P ($n = 12$; ▲). Values represent mean±SE.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

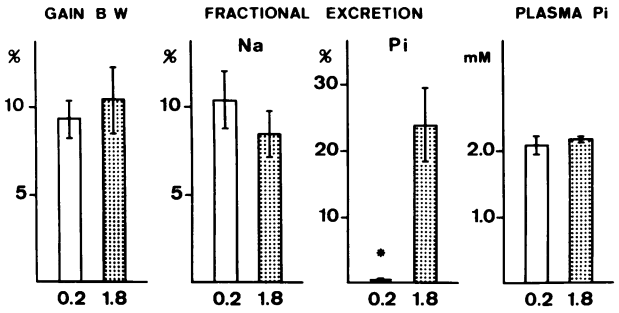

FIGURE 2 Maintenance of the diet-induced change in the tubular handling of P_i during marked ECVE. Fractional excretions of sodium and P_i were measured in rats pair-fed diets containing either 0.2 g/100 g P (open bars) or 1.8 g/100 g P (dotted bars) when infused i.v. with a solution of 0.15 mM NaCl at a rate of 20 ml/h for 195 min. The gain in body weight (BW) achieved at the end of the clearance experiment is expressed as the percent of the body weights at the onset of the clearance experiment. They were: 176±2 g ($n=5$) and 193±3 g ($n=6$) for the group fed 0.2 and 1.8 g/100 g P diet, respectively. Values represent mean±SE. *$P < 0.001$.

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_i/FLP_i) was always higher in rats fed the low P_i (A) than in those fed the high P_i diet (C) for any given $[P_i]_{PI}$ 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. UVNa/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 UVNa/GF than in UVP_i/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 $[P_i]_{PI}$, fractional excretion of P_i ($UVP_i/FLP_i \times 100$) 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). C_{in} 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 $[P_i]_{PI}$ 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 $[P_i]_{PI}$ significantly lower than those fed control or high-P_i diets ($P < 0.001$). Since C_{in} was about the same in all three groups, FLP_i was also lower in group A as compared to groups B and C ($P < 0.001$). Again UVP_i and UVP_i/FLP_i were related to the previous dietary intake of P_i. Thus, although FLP_i was somewhat higher after 1.2 g/100 g P (group B) than in the other two groups, UVP_i and UVP_i/FLP_i 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 $[P_i]_{PI}$ in group A than in group C ($P < 0.001$), but FLP_i were comparable. Nev-

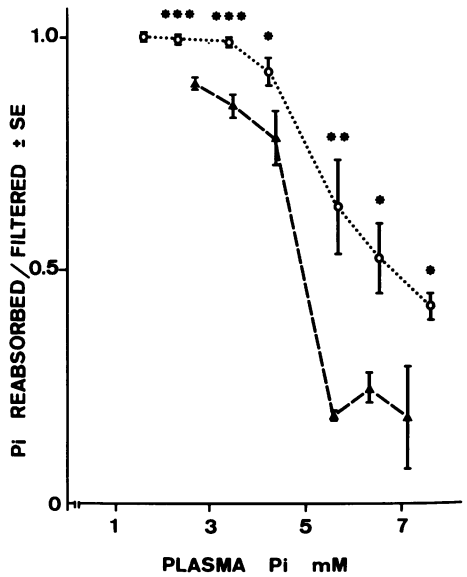

FIGURE 3 Fractional reabsorption of P_i determined under acute sodium chloride and stepwise-increasing sodium phosphate infusions (4 ml/h as described in Methods) in 28 TPTX rats pair-fed diets containing either 0.2 g/100 g P ($n=16$: ○) or 1.8 g/100 g P ($n=12$; ▲). Values represent mean±SE. Data obtained between each millimolar unit of plasma P_i concentration were pooled. *$P < 0.05$; ** $P < 0.02$; ***$P < 0.001$.

TABLE III

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

Group	Dietary P	n	Body wt	Period	C_{In}	V	$[P_i]_{PI}$	FLP_i	UVP_i	UVP_i/FLP_i	TRP_i	UVN_a	$[Ca]_{PI}$
	g/100 g		*g*		*ml/min*	*μl/min*	*mM*	*μmol/min*	*μmol/ml GF*	*×100*	*μmol/ml GF*	*μmol/ml GF*	*mM*
A	0.2	6	158 ±4	I	1.24 ±0.04	47 ±4	1.57§ ±0.02	1.94§ ±0.08	0.0018§ ±0.0006	0.115§ ±0.037	1.57§ ±0.03	4.95 ±0.38	2.42§ ±0.06
				II	1.32 ±0.14	27 ±6	6.59 ±0.07	8.65 ±0.91	4.25* ±0.13	64.6 ±1.2	2.33 ±0.12	7.64 ±0.82	1.00 ±0.02
B	1.2	4	174 ±9	I	1.42 ±0.09	49 ±8	3.15 ±0.15	4.50 ±0.46	0.081 ±0.039	2.45 ±1.1	3.07 ±0.11	5.11 ±0.57	1.52 ±0.06
				II	1.51 ±0.10	50 ±8	6.36 ±0.39	9.52 ±0.45	4.80 ±0.22	76.20 ±5.5	1.56 ±0.46	8.17 ±0.60	1.02 ±0.04
C	1.8	5	173 ±6	I	1.34 ±0.13	34 ±3	2.82 ±0.14	3.76 ±0.36	0.399§ ±0.083	13.76§ ±2.2	2.42§ ±0.06	5.13 ±0.69	1.71 ±0.06
				II	1.49 ±0.05	57 ±10	5.81* ±0.18	8.67 ±0.38	4.48 ±0.13	78.0 ±2.75	1.33 ±0.18	7.89 ±0.79	1.12 ±0.16

Thyroparathyroidectomy 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, UVP_i and UVP_i/FLP_i were lower in group A than in group C ($P < 0.01$ for UVP_i/FLP_i). As observed in the intact rats, TPTX animals fed a low P_i diet responded to the same intravenous load of P_i differently from animals on higher P_i intake. TRP_i/GF slightly increased in group A, whereas it markedly decreased in the rats fed higher P_i diets. This different response led to a significantly lower TRP_i/GF in group C as compared to group A ($P \leq 0.001$).

Results from these clearance experiments and of those performed with stepwise-increasing i.v. loads of P_i are shown in Fig. 3. TRP_i/FLP_i measured at $[P_i]_{PI}$ ranging from 1 to 7.5 mM in rats was always higher for a given $[P_i]_{PI}$ in the animals fed the low-P_i diet (A) than in those receiving the high-P_i diet (C).

As in the intact rats, there was no significant difference in UVNa/GF between rats fed the low or high P_i diet as compared to the control group during both periods I and II.

The calcemia per se has been implicated in the regulation of P_i 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 $[Ca]_{PI}$ for a given $[P_i]_{PI}$ (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-P_i diets for 10 days. The effectiveness of parathyroidectomy was assessed 4 days after surgery: $[Ca]_{PI}$ 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-P_i diet, respectively. The results of the clearance experiment are shown in Table IV. In the unloaded state (period I), at comparable $[P_i]_{PI}$ and FLP_i, UVP_i/FLP_i was significantly lower in the animals fed the low-P_i diet (D) than in those fed the high-P_i diet (E). Under intravenous loading with P_i (period II), $[P_i]_{PI}$ was not significantly different between the two dietary groups. Although FLP_i was higher in the group on low P_i intake (D), its $UVP_i/$

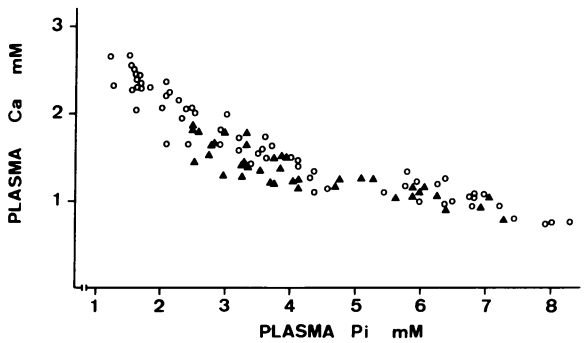

FIGURE 4 Calcemia determined at various plasma concentrations of P_i in TPTX rats pair-fed diets containing either 0.2 g/100 g P (○) or 1.8 g/100 g P (▲). The fractional reabsorption of phosphate in these rats is presented in Fig. 3.

TABLE IV

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

Group	Dietary P	n	Body wt	Period	C_{In}	$[P_i]_{PI}$	FLP_i	UVP_i/FLP_i	TRP_i	UV_{Na}
	g/100 g		*g*		*ml/min*	*mM*	*μmol/min*	*×100*	*μmol/ml GF*	*μmol/ml GF*
D	0.2	4	211 ±9	I	1.29 ±0.08	2.48 ±0.30	3.11 ±0.23	0.6‡ ±0.05	2.48 ±0.30	6.99 ±1.57
				II	1.17 ±0.04	6.59 ±0.38	7.66* ±0.42	65.3* ±1.7	2.27‡ ±0.12	11.82 ±0.82
E	1.8	5	199 ±7	I	1.20 ±0.06	2.78 ±0.08	3.37 ±0.25	7.7 ±1.7	2.60 ±0.07	6.60 ±1.43
				II	0.97 ±0.04	6.61 ±0.69	6.40 ±0.26	77.8 ±4.3	1.49 ±0.30	11.72 ±0.45

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.

FLP_i was significantly lower than in the group on high P_i 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 P_i loading was also different according to the prior dietary intake of P_i. It led to a significantly lower TRP_i/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 P_i 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 P_i 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 P_i 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 P_i between low and high P_i-fed animals. This was measured at similar $[P_i]_{PI}$ (low 3.19±0.26, $n = 5$; high 3.38±0.41 mM, $n = 4$), C_{In} (1.31±0.06 and 1.29±0.09 ml/min), and FLP_i (4.18 ±0.43 and 4.42±0.75 μmol/min). In group A, prolonging the low-P_i diet led to a decrease in $[P_i]_{PI}$ (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 P_i decreased with time; $[P_i]_{PI}$ 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 $[P_i]_{PI}$ to its initial value might be related to the further fall in the tubular reabsorption occuring 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, $[Ca]_{PI}$ 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) P_i

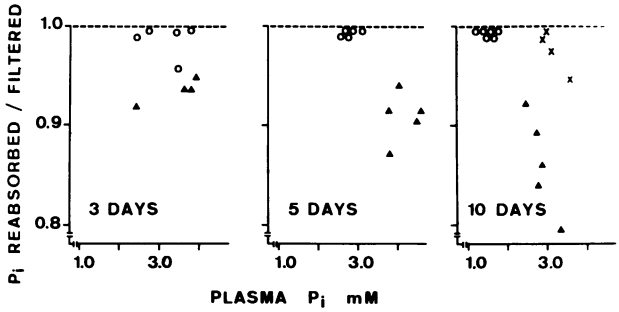

FIGURE 5 Fractional reabsorption of P_i in TPTX rats pair-fed diets containing either 0.2 g/100 g P (○) or 1.8 g/100 g P (▲) for 3, 5, or 10 days. The data obtained with a 1.2 g/100 g P diet (×) are also presented. Values at 10 days correspond to those given in Table III. Thyroparathyroidectomy was done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

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, $[P_i]_{PI}$ was lower after P_i deprivation (low, 2.77±0.12, $n=8$; high, 3.41±0.06 mM, $n=6$; $P<0.01$). But because C_{In} was slightly higher in this group (low, 1.51±0.07; high, 1.29±0.09 ml/min, NS), both groups displayed the same FLP_i (low, 4.19±0.24; high, 4.40±0.28 μmol/min). In this condition, as in the preceeding series, a marked difference in the fractional reabsorption of P_i was observed between the two dietary groups. The animals fed the low-P_i diet reabsorbed virtually all P_i filtered (99.3±0.3%), whereas the rats on high P_i intake reabsorbed only 95.2±1.0% ($P<0.01$). Thus it appears that the adaptive response of the tubule to dietary P_i 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 P_i intake by modulating its capacity to reabsorb P_i. Indeed, the diet-induced change in the fractional reabsorption of P_i exceeds that expected merely from variations in the filtered load of P_i. The capacity of the tubule to reabsorb P_i varies according to homeostatic requirements, being greater under a low- than under a high-P_i 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-P_i diets. However, it diminishes the ability of the renal tubule to adapt to a high P_i intake. Indeed, the capacity of the tubule to reabsorb P_i remains greater in TPTX rats fed high-P_i diets than in intact rats (Tables II and III). This might be responsible for their higher $[P_i]_{PI}$. The response to an acute infusion of P_i differs markedly according to the preceeding P_i intake in both intact and TPTX rats. In the intact animals fed a low P_i 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 P_i (TmP_i) across the tubular epithelium over a wide range of plasma phosphate concentrations. In intact rats fed a high P_i diet the acute elevation of plasma P_i led to a sharp decrease of the tubular reabsorption. Therefore under these conditions no apparent TmP_i could be observed, confirming previous observations in rats (23, 24). Thus the demonstration of a TmP_i 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 TRP_i/GF to values not significantly different from 0 in some rats fed a high-P_i diet (Table II) suggests strongly the existence of a secretory flux of P_i 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 P_i 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 P_i. The higher plasma calcium concentration observed in TPTX rats fed the low P_i diet could be directly related to the higher fractional reabsorption displayed by these animals under NaCl infusion. However, under P_i infusion the difference in the tubular capacity to transport P_i was observed with no difference in $[Ca]_{PI}$ for any given $[P_i]_{PI}$. 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 P_i.

ECVE has been shown to decrease the tubular reabsorption of P_i 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 P_i induced by the dietary intake of P_i 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 P_i 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 $[P_i]_{PI}$ in our rats on low P_i diet. This finding, however, cannot explain the greater tubular reabsorption observed in the rats fed the low P_i diet: indeed, alkalinization of urine has been shown to decrease the tubular reabsorption of P_i in dogs and in man (29–31). Furthermore, the urinary pH was the same in both groups during the acute load with P_i, while the difference in the handling of P_i still occurred.

Our data show that the diet-induced modulation of the renal tubular capacity to transport P_i is in part independent of PTH, of $[Ca]_{PI}$ at the time of the clearance measurement, of ECVE, and of urinary pH. It is possible that vitamin D_3 metabolites could be among the factors involved in the tubular adaptation. Indeed some of the active metabolites of vitamin D_3 have been shown to enhance the renal reabsorption of P_i (32, 33). Furthermore, P_i 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 P_i diet (0.05 g/100 g) excreted virtually no P_i 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 P_i diet were reported to reabsorb practically all P_i 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 P_i in response to dietary P_i. It indicates the existence of an as yet unknown regulatory factor involved in the urinary excretion of P_i. The exact mechanism underlying this renal tubular adaptation phenomenon as well as its localization along the nephron remain to be elucidated.

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