ADAPTATION IN RENAL PHOSPHORUS EXCRETION UNDER
THE INFLUENCE OF PARATHYROID; A STUDY IN
URETERALLY CATHETERIZED RATS

MIKIO SHIKITA, SUSUMU TSURUFUJI AND YOSOJI ITO

Department of Physiological Chemistry, Faculty of Pharmaceutical
Sciences, University of Tokyo, Tokyo

Relation of parathyroid glands to the renal excretion of phosphorus has been
the subject of many earlier investigations in both humans and experimental ani-
imals since the classic observation of Greenwald (1911) on parathyroidectomized
dogs. Albright and his co-workers (1929) proposed a concept that the parathyroids
act primarily on renal tubules and decrease the net reabsorption of phosphorus.
On the other hand, many others claimed that the parathyroids have a direct
action on bones (Barnicot, 1948; Chang, 1951; Gaillard, 1955) and that parathyroid
extract (PTE) has an effect on calcium metabolism even in nephrectomized ani-
mals (Stewart and Bowen, 1951; Grollman, 1954 and many others). Albright's
hypothesis was criticized, furthermore, from a chemical (Neuman and Neuman,
1953) or clinical (Coxon, 1954; Lubell, 1957 and many others) point of view.
Handler et al. (1951) and others (Japhan and Pitts, 1948; Hogben and Bollman,
1951) reported that PTE increased renal glomerular filtration rate and Stewart
and Bowen (1952) attributed the phosphaturic activity of PTE to a nonspecific
action of artifacts in the hormone preparation. However, all the facts previously
observed by many workers can not always be explained by the action of the
hormone only on bones. There are increasing evidences that the parathyroid
hormone has a direct effect not only on bones but also on renal tubules. Elabo-
rate experiments were performed by Levinsky and Davidson in chicks (1957) and
Nicholson in dogs (1959), followed by Beutner and Munson (1960) who reported
immediate changes in urinary phosphorus excretion in rats after parathyroidecto-
my or after the injection of PTE and re-evaluated the clinical observation of
Albright et al. (1929). Furthermore, Samiy et al. (1960) and Pullman et al. (1960)
demonstrated a direct action of the hormone on the renal tubules in dogs using
a highly purified preparation provided by Rusmussen and Craig (1961). On the
other hand, Foulks and Perry (1959) reported a marked decrease in GFR
following parathyroidectomy in dogs. They could find no significant difference
between the intact and parathyroidectomized dogs in regard to the renal
threshold for phosphorus excretion, the phosphate Tm, etc., concluding that
hyperphosphatemia caused by parathyroidectomy is not due to the decrease in
urinary phosphorus excretion but to the decreased uptake of phosphorus by body
cells. In the present study, the authors observed urinary phosphorus excretion
after acute intravenous injection of inorganic phosphate in parathyroidectomized

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or intact rats which were fed on a low phosphorus diet, and examined if kidneys under the influence of parathyroids can adapt to the changes in serum phosphorus level.

**EXPERIMENTAL**

**Phosphaturia after an acute intravenous injection of inorganic phosphate in rats fed a low phosphorus diet**

**Experiment A**

Twelve female rats of Wistar-Imamichi strain, weighing 180 to 208 g, 3-months-old, were used. A half of them were fed on a diet of low phosphorus content (LP-1 diet), and the other half on a normal control diet (N-1 diet) for the preceding 2 days. Compositions of these diets are shown in Table 1. The animals were anesthetized by Nembutal injection, ureterally catheterized as described in the preceding paper (Shikita, 1962), and 250 mins. later injected through the femoral vein with 0.5 ml/100 g body weight of inorganic phosphate solution* labelled with $^{32}$P. By this injection 0.93 mg/100 g body weight of phosphorus was given in the low phosphorus diet group and 0.14 mg in the control diet group. As to the radiophosphorus, the same amount of $^{32}$P (100 μCi/100 g body weight) was injected in each rat. It took 1 min. to inject the phosphate solution. Accurately 5 mins. after the start of the injection, carotid artery was cut and the blood

<table>
<thead>
<tr>
<th>Table 1. The composition of the diets</th>
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<table>
<thead>
<tr>
<th></th>
<th>Normal control diet</th>
<th>Low phosphorus diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-1</td>
<td>N-2</td>
</tr>
<tr>
<td>Corn starch Dextrin</td>
<td>53.0 (g)</td>
<td>54.0</td>
</tr>
<tr>
<td>Milk casein</td>
<td>25.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>~</td>
<td>15.0</td>
</tr>
<tr>
<td>Filter paper powder</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>CaCO$_3$</td>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Yeast extract 10% sol.</td>
<td>3.0 (mL)</td>
<td>3.0</td>
</tr>
<tr>
<td>Salt mixture sol.*</td>
<td>20.0</td>
<td>20.0</td>
</tr>
<tr>
<td>KH$_2$PO$_4$ 15% sol.</td>
<td>15.0</td>
<td>12.0</td>
</tr>
<tr>
<td>KCl 10% sol.</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>FeCl$_3$ 29% sol.</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Chocola A**</td>
<td>1 drop</td>
<td>1</td>
</tr>
</tbody>
</table>

* Salts mixture solution; NaCl 6.5 g, MnSO$_4$·4H$_2$O 0.10 g, MgSO$_4$·7H$_2$O 2.5 g, CuSO$_4$·5H$_2$O 0.060 g, in 100 mL aqueous solution.

** Chocola A; Eisai Co. Ltd. (Tokyo) Each mL contains 30,000 i.u. of Vitamin A and 3,000 i.u. of Vitamin D$_2$

* Phosphate injection; Na$_2$HPO$_4$, KH$_2$PO$_4$ 4:1, pH 7.4, made isotonic by adding NaCl if necessary
was withdrawn into centrifuging tubes for the following 1 min. Serum phosphorus concentration in the low phosphorus diet group at the time of sacrifice was 1.14 times that in the controls, and the difference was statistically significant at 0.05% level (Table 2). It is to be recalled that the formers received the injection of larger amount of phosphorus. Despite the higher concentration of phosphorus in the serum, the phosphorus-deficient rats excreted into their urine far less amount of phosphorus than the normal controls. When compared in terms of phosphorus clearance (phosphorus excretion divided by serum phosphorus level), there is a highly significant difference between the 2 groups (P<0.001) (Table 2). As to the renal clearance of radiophosphorus, the difference between them was also highly significant (P<0.001).

**Experiment B**

Eight female rats of Wistar-Imamichi strain, 3.5-months-old, weighing 200 to 237 g, were divided into 2 groups and placed on a low phosphorus diet (LP-1 diet) or on the normal control diet (N-I diet) for 2 days as in the preceding experiment. They were ureterally catheterized and received an intravenous injection of 1.0 ml of 0.1 M phosphate buffer. Two hrs. later they were injected again with the same amount of the phosphate. As shown in Figure 1, after the 1st injection of the phosphate solution, immediate hyperphosphaturia was observed in the control rats, but not in the rats of low phosphorus diet group. This result is consistent with that observed in Experiment A. Three hrs. after the 2nd injection, phosphorus excretion as well as urinary flow became constant rate in both groups. At that time the rats were sacrificed by exsanguination from the carotid arteries. Serum phosphorus concentration was 6.41 mg/dl in the controls and 7.77 mg/dl in the low phosphorus diet group. Higher level in serum phosphorus was found rather in the latter animals, in spite of the fact that they had been fed on the low phosphorus diet and injected with the same amount of

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<table>
<thead>
<tr>
<th>Diet</th>
<th>Low phosphorus (LP-1)</th>
<th>Normal control (N-I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Phosphorus injected (mg/100 g body weight)</td>
<td>0.93</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum phosphorus (g/ml)</td>
<td>77.6 ±2.9</td>
<td>68.0 ± 1.7</td>
</tr>
<tr>
<td>Serum 32p (% dose/ml)</td>
<td>1.15±0.12</td>
<td>1.05± 0.04</td>
</tr>
<tr>
<td>Urinary flow (µl/min.)</td>
<td>15.7 ±2.9</td>
<td>27.2 ±10.8</td>
</tr>
<tr>
<td>Urinary phosphorus (g/min.)</td>
<td>2.0 ±0.4</td>
<td>23.1 ± 2.0</td>
</tr>
<tr>
<td>Urinary 32p (% dose/min.)</td>
<td>0.07±0.02</td>
<td>0.80± 0.05</td>
</tr>
<tr>
<td>P clearance (µl/min)</td>
<td>25 ±4</td>
<td>340 ± 22</td>
</tr>
<tr>
<td>32p clearance (µl/min)</td>
<td>60 ±4</td>
<td>755 ±11</td>
</tr>
<tr>
<td>32p clearance/P clearance</td>
<td>2.4 ±0.6</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>32p in Kidneys (% dose)</td>
<td>1.73± 0.06</td>
<td>2.61± 0.16</td>
</tr>
</tbody>
</table>
phosphate as the controls. Phosphorus clearance at the time of sacrifice was calculated to be 554 μl/min. for the control and 327 μl/min. for the low phosphorus diet group. The difference between the 2 groups was significant in the probability of 0.05. Similar to the result of Experiment A, the low rate in the renal clearance of phosphorus may well explain the paradoxical-looking hyperphosphatemia in the animals of low phosphorus diet group.

It should be noted in Experiment B that repeated injections of phosphate resulted in a significant increase in phosphorus clearance in both groups of rats. Especially, phosphorus clearance in the low phosphorus diet group in Experiment B amounted to as much as 1300% of that observed in Experiment A.

**Effect of parathyroidectomy on serum calcium and phosphorus level as influenced by dietary phosphorus intake**

As shown in Table 3, low phosphorus intake resulted in a decrease in serum phosphorus accompanied by an increase in serum calcium. These changes in serum calcium and phosphorus make a marked contrast to the changes in parathyroidectomy (Talmage and Kraintz, 1954; Munson, 1955 and many others). Accordingly, the combined effect of parathyroidectomy and low phosphorus intake on serum phosphorus and calcium was studied. Two groups of male rats of Wistar strain, 2-months-old, were housed in individual cages. A half of them were parathyroidectomized and the other half sham operated and served as controls. They were fed on the normal diet (N-4 diet) for 4 days after the operation, then each group was further divided into 2 subgroups which consisted of the normal diet and very low phosphorus diet (LP-4 diet) groups respectively. After
Table 3. Effect of low phosphorus intake on serum inorganic phosphorus and calcium concentration in rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>P content of the diet</th>
<th>Days on the diet</th>
<th>No. of animals</th>
<th>Serum phosphorus (mg/dl)</th>
<th>Serum calcium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-2</td>
<td>0.510 (% )</td>
<td>4</td>
<td>7</td>
<td>8.2±0.3</td>
<td>9.6±0.1</td>
</tr>
<tr>
<td>LP-2</td>
<td>0.101</td>
<td>4</td>
<td>7</td>
<td>6.8±0.3 **</td>
<td>10.5±0.2 **</td>
</tr>
<tr>
<td>N-3</td>
<td>0.546</td>
<td>2</td>
<td>6</td>
<td>9.9±0.2</td>
<td>9.6±0.1</td>
</tr>
<tr>
<td>LP-3</td>
<td>0.205</td>
<td>2</td>
<td>6</td>
<td>8.2±0.4 **</td>
<td>10.3±0.1 ***</td>
</tr>
</tbody>
</table>

The difference between low phosphorus diet group and the corresponding control group is statistically significant at 1% (**) or 0.1% (***) level.

Table 4. Relation of dietary phosphorus intake to the effect of parathyroidectomy on serum calcium and phosphorus level in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diet</th>
<th>Initial 4 days</th>
<th>Following 5 days</th>
<th>No. of animals</th>
<th>Serum calcium (mg/dl)</th>
<th>Serum phosphorus (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>N-4</td>
<td>N-4</td>
<td></td>
<td>6</td>
<td>9.6±0.2</td>
<td>9.6±0.4</td>
</tr>
<tr>
<td>PTX</td>
<td>N-4</td>
<td>N-4</td>
<td></td>
<td>6</td>
<td>7.1±0.6</td>
<td>13.2±0.8</td>
</tr>
<tr>
<td>Sham</td>
<td>N-4</td>
<td>LP-4</td>
<td></td>
<td>6</td>
<td>11.5±0.1</td>
<td>6.3±0.3</td>
</tr>
<tr>
<td>PTX</td>
<td>N-4</td>
<td>LP-4</td>
<td></td>
<td>6</td>
<td>11.6±0.2</td>
<td>6.1±0.5</td>
</tr>
</tbody>
</table>

Sham: Sham-operated, PTX: Parathyroidectomized.

5 days feeding on these diets, all of the rats were sacrificed. In the low phosphorus diet group, both parathyroidectomized and sham-operated rats showed a similar increase in serum calcium and a similar decrease in phosphorus, compared with sham-operated normal diet group (Table 4). Statistical analysis showed that the effect of diet, parathyroidectomy and their interaction are all significant at 1% level. In parathyroidectomized rats, serum calcium must have been decreased and serum phosphorus increased during initial 4 days feeding on the normal diet. Thus, it is suggested that the low phosphorus intake for the following 5 days suppressed the effect of parathyroidectomy, the serum phosphorus level which had been elevated being lowered progressively to the subnormal level, and vice versa as to the serum calcium.

Phosphaturia after acute intravenous injection of inorganic phosphate in parathyroidectomized rats

Female rats of Donryu strain, aged 3 months, were used. A half of them were parathyroidectomized and the other half sham operated. All the animals were kept on the low phosphorus diet (LP-1 diet) for the following 2 days. Then, each group was further divided into 2 subgroups and one subgroup sacrificed without further treatment. The other subgroup were ureterally catheterized as described previously (Shikita, 1962) and injected with the phosphate solution. The results are shown in Figure 2 and Table 5. In the rats killed without injection
Fig. 2. Urinary phosphorus excretion following the intravenous injection of inorganic phosphate in parathyroidectomized or sham-operated rats

All the animals had been kept on a low phosphorus diet for the preceding 2 days and injected with 3.1 mg/rat of phosphate at each time indicated by an arrow.

Table 5. Serum calcium and phosphorus levels with or without intravenous injection of inorganic phosphate in parathyroidectomized (PTX) or sham-operated rats (Sham)

<table>
<thead>
<tr>
<th></th>
<th>No injection of P</th>
<th>After injection of P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>Sham</td>
<td>11.2±0.1 (4)</td>
</tr>
<tr>
<td></td>
<td>PTX</td>
<td>11.5±0.3 (4)</td>
</tr>
<tr>
<td>Serum phosphorus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>Sham</td>
<td>8.2±0.5 (4)</td>
</tr>
<tr>
<td></td>
<td>PTX</td>
<td>8.4±0.4 (4)</td>
</tr>
</tbody>
</table>

All animals had been kept on low phosphorus diet for the preceding 2 days. Figures in parenthesis represent the number of the animals.

Fig. 3. A secondary increase in phosphorus excretion following the acute intravenous injection of inorganic phosphate

A female rat, 191 g, 3-months-old, fed on a low phosphorus diet for the preceding 2 days, ureterally catheterized and injected with 2 ml of 0.1 M phosphate buffer in 2 divided doses. The dotted line represents the urinary flow rate.
of phosphate, there found no effect of parathyroidectomy on serum calcium and phosphorus. On the other hand, in the rats which were injected with phosphate, there was a significant difference in serum calcium and phosphorus ($p<0.01$) between parathyroidectomized and sham-operated rats. These are consistent results with that obtained in the foregoing experiment in which parathyroidectomy failed to influence serum calcium and phosphorus in the low phosphorus diet group (Table 4). It can be concluded, therefore, that the effect of parathyroidectomy which had been masked by low phosphorus intake became apparent only after the injection of phosphate. It seems probable that hypophosphaturia in parathyroidectomized rats makes a contribution to the development of hyperphosphatemia and hypocalcemia in them.

DISCUSSION

Table 2 shows that the renal clearance of radiophosphorus was more than twice as much as that of phosphorus. It is necessary to elucidate this discrepancy of the excretion rates. Since injected radiophosphorus emigrates very rapidly from the blood vessels into the extravascular spaces, radiophosphorus concentration of blood serum decreases very rapidly. Accordingly, radiophosphorus concentration in glomerular filtrate should fall very fast in parallel with its fall in the blood. In order to obtain exact clearance of radiophosphorus, therefore, $\frac{32P_u}{t} \int_0^t \frac{(32P)_s \cdot dt}{t}$ must be calculated, where $32P_u$ means an amount of radiophosphorus excreted and $(32P)_s$ means the concentration of radiophosphorus in the serum. In the present study $32P_u$ was actually determined, but $\int_0^t \frac{(32P)_s \cdot dt}{t}$ was never measured, and in place of a true value, the radiophosphorus concentration of the serum at the time of sacrifice was used in the calculation. As clearly understood, $\int_0^t \frac{(32P)_s \cdot dt}{t}$ should be larger than the radiophosphorus concentration at the time of sacrifice, therefore, so-computated, apparent clearance of radiophosphorus would be greater than a true clearance, whereas in the calculation of the clearance of inorganic phosphorus $\int_0^t (P)_s \cdot dt$ would not very much differ from the serum concentration of phosphorus at sacrifice. Accordingly, it is not strange that the clearance value of radiophosphorus in Table 2 exceeds that of phosphorus.

Govaert (1947, 1952 and 1954) suggested in his reports that phosphate exists in 2 forms in the blood; an easily filtrable, excretable form and an unfiltrable, non-excretable one. Handler and Cohen (1951 and 1953), however, re-examined the experiment of Govaert and denied the existence of unfiltrable form of inorganic phosphate in the serum. In the preceding report (Shikita, 1962) one of the present authors also denied the significant existence of unfiltrable form of inorganic phosphorus even in the case of phosphorus-deficient or para-
thyroidectomized rats. Therefore, it is unreasonable to suppose that injected phosphorus had not been excreted in the rats of low phosphorus diet group because of its immediate transformation to an unfiltrable complex in their blood, and the present experiments actually showed that the renal clearance of phosphorus was lowered by feeding the animals on the low phosphorus diet and that the repeated injections of phosphate in them resulted in an increase in the clearance. Anyway, it is clear that the rate of phosphorus excretion is not directly proportional to the serum phosphorus concentration. These observations may be regarded as the evidences that there is an adaptive change in the renal threshold for the excretion of phosphorus and that it requires several hours to settle a new threshold as shown in Figures 1 and 3. There was a lag of half an hour or more before starting continuous hyperphosphaturia following the acute intravenous injection of phosphate. A typical case is shown in Figure 3. It is apparent that the increase in phosphorus excretion is not due to an increase in the urinary flow rate. The observation shown in Figure 3 may be an additional evidence for the renal adaptation in response to the excess or low phosphorus intake. Neuman and Chen (1953) observed a similar delay in renal calcium excretion after an intravenous injection of calcium in dogs.

Interestingly, parathyroidectomy caused neither hypocalcemia nor hyperphosphatemia in phosphorus deficient rats (Tables 4 and 5). This observation is consistent with that reported by Thompson and Hiatt (1957) that oral administration of aluminium hydroxide could lower the serum phosphorus level even in hypoparathyroid patients. Furthermore, it is of deep interest that the intravenous injection of inorganic phosphate in the rats fed on low phosphorus diet caused hypocalcemia only in the absence of parathyroid glands (Table 5). The observation that the injection of phosphate caused hyperphosphatemia without a decrease in serum calcium has been reported by Munson (1955) as one of the evidences against the “solubility product concept” of Albright. In the present experiment, however, the injection of the phosphate caused more hyperphosphatemia in the parathyroidectomized rats than in the controls, and hypocalcemic effect was observed only in the formers. These observations seem to be explicable by a postulation that the parathyroids have a phosphaturic action on kidneys besides the calcium mobilizing action on bones and that the secretion of phosphaturic hormone is diminished or enhanced, respectively, in response to hypophosphatemia caused by low phosphorus intake or to hyperphosphatemia caused by the injection of inorganic phosphate.

It has already been suggested by Albright and his coworkers that an increase in serum phosphorus stimulates the parathyroids function (1929). There are some other workers who supported this concept (Thompson and Hiatt, 1957; Crawford et al. 1950; Goldman and Basett, 1958). However, as it was criticized by Fourman (1960), it might be hypocalcemia caused by the administration of phosphate and not the hyperphosphatemia itself that stimulates the parathyroids, for it has been suggested by many workers that hypocalcemia stimulates the parathyroids function (Patt and Luckhardt, 1942; Buckner and Nellor, 1960 and many others). In the present experiment, however, hyperphosphaturia was induced by the intravenous injection of phosphate in the intact rats placed on the low phosphorus diet,
though hypocalcemia was never induced. Therefore, it seems probable that it is the hyperphosphatemia but not the hypocalcemia that stimulated the parathyroid glands of these animals.

SUMMARY

1. The rats placed on low phosphorus diet excreted less phosphorus in the urine than control rats, though serum phosphorus level in the phosphorus-deficient rats was elevated higher than the normal level by an intravenous injection of inorganic phosphate.

2. Repeated intravenous injections of inorganic phosphate into phosphorus-deficient rats resulted in hyperphosphaturia, though serum phosphorus remained at the same or rather lower level than before the injection.

3. It was often observed that there was a lag of 30 mins. or more before starting a continuous phosphaturia induced by an acute intravenous injection of inorganic phosphate.

4. Low phosphorus intake caused a significant increase in serum calcium concentration.

5. Parathyroidectomy caused no hypocalcemia nor hyperphosphatemia in the rats fed on low phosphorus diet.

6. Effects of parathyroidectomy which had been masked by low phosphorus intake became apparent shortly after the injection of the phosphate. The injection caused less phosphaturia and more hyperphosphatemia in the parathyroidectomized rats than in the control rats, resulting in a decrease in serum calcium only in the formers but not in the latters.

7. These results seem to suggest that there is an adaptive change in the renal threshold for phosphorus excretion in response to excess or low phosphorus intake, and that the parathyroids are involved in the mechanism of the adaptation.

REFERENCES

Handler, and D. V. Cohen. Metabolic Interrelations; Transactions of the Fifth Conference.