Phosphate Excretion in the Parathyroidectomized Rat Receiving Parathyroid Hormone

By John C. Van Stone and Jessie E. Hano

The relationship of phosphorus excretion to phosphorus intake was studied in normal, parathyroidectomized, and thyroparathyroidectomized rats. Parathyroidectomized rats were given constant parathyroid hormone (PTH) replacement. Thyroidectomized rats received, in addition to PTH, thyroxine replacement. The experimental animals were able to conserve and excrete phosphorus as well as the normal animals. Morning serum phosphorus did not change in any of the groups despite a fivefold increase in phosphorus intake.

PHOSPHORUS EXCRETION BY THE KIDNEYS varies with parathyroid activity. However, several observations suggest that there are also other factors controlling phosphorus balance. Hypoparathyroid patients, whose serum calcium is maintained normal with either intravenous calcium or pharmacologic doses of Vitamin D, can increase phosphorus excretion in response to an increased phosphorus intake without an increase in serum phosphorus.1,2 Patients with hyperparathyroidism can decrease phosphorus excretion in response to a low phosphorus diet.3,4 Similarly, they can increase phosphorus excretion when given a phosphorus challenge.5 They do this rapidly with minimal changes in serum phosphorus concentration. If it is assumed that parathyroid tissue autonomously functions in hyperparathyroidism (which may5 or may not6 be a valid assumption), and is either absent or continuously functioning maximally in hypoparathyroidism (which is also a questionable assumption), then some nonparathyroid-mediated regulation of phosphorus excretion must be present. This would not be unexpected, however, since other ions such as sodium,7 calcium,8 and possibly potassium9 have more than one mechanism controlling their concentration.

We have studied phosphorus balance in adult rats that have been parathyroidectomized or thyroparathyroidectomized and given a constant parathyroid (PTH) replacement.

MATERIALS AND METHODS

Adult white Sprague-Dawley rats weighing 250–500 g were placed on a low phosphorus diet (rat low phosphorus diet, Nutritional Biochemicals) for the entire period of study. This diet contains 0.13% phosphorus and 0.23% calcium, with the other constituents essentially the same as a regular laboratory rat diet. After 3 days to 1 wk on this diet, parathyroidectomy by cautery or thyroparathyroidectomy by excision and cautery were performed with

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Received for publication February 22, 1972.

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Metabolism, Vol. 21, No. 9 (September), 1972
the use of an operating microscope. Blood calcium was determined on the 4th, 8th, and 12th postoperative days, and all animals with a serum calcium above 8 mg/100 ml were rejected from the study. Control rats were placed on the diet, but were not operated on. The parathyroidectomized group (PTX) consisted of seven animals, and there were five animals each in the thyroparathyroidectomized group (TPTX) and control group (CG). After adequacy of the parathyroidectomy was established, the PTX animals received 20 units of bovine parathyroid hormone (Parathormone, Eli Lilly) subcutaneously every 12 hr. The TPTX animals received the same dose of PTH plus 15 μg of thyroxine (Synthyroid, Flint) every 3 days. The control animals were not injected. Blood specimens were obtained between 10:00 a.m. and noon on lightly ether anesthetized animals every 4-7 days by retroorbital puncture. The animals were kept in separate metabolic cages and urine collected in 48-hr periods. The food intake was measured daily and from this the phosphorus intake calculated. After 8 days on this diet with hormone replacement, 8 mg of phosphorus/100 g body weight were given by gastric tube at 5:00 p.m. daily as a neutral sodium potassium phosphate solution for the next 8 days. This was followed by 8 day periods in which the phosphorus supplement was increased to 16 mg/100 g and then 32 mg/100 g. The added phosphorus was then stopped and 20 cc of CaCO$_3$ suspension (Basaljel, Wyeth) added to every 100 g of the diet. Diarrheal contamination of the urine collections was produced by the 32-mg phosphorus diet, and this period was dropped from the study. The serum specimens were analyzed by the AutoAnalyzer simultaneously for phosphorus, calcium, and creatinine on less than 0.2 ml of serum. Urine was analyzed for phosphorus and creatinine. The 48-hr creatinine excretion was used to test the adequacy of the urine collections. Any urine specimen containing two standard deviations less than the mean creatinine excretion for that particular animal was discarded. Less than 5% of the specimens were eliminated from each group. Body surface area was calculated from the formula of Benedict. Calcium was determined fluorometrically by the method of Hill. Phosphorus and creatinine were determined by standard AutoAnalyzer methods.

RESULTS

The serum calcium was normal on the low phosphorus diet in all groups and decreased significantly with phosphorus loading ($p<0.001$) to slightly subnormal values in both experimental groups (Fig. 1). It did not significantly change in the control group. Serum calcium returned to normal levels when phosphorus supplementation was stopped and CaCO$_3$ was added to the diet.

The serum phosphorus was significantly higher throughout the study in both experimental groups when compared to the control group (Fig. 1): CG vs. TPTX $p<0.001$; PTX vs. CG $p<0.05$. However, within each group there was no significant change in serum phosphorus with phosphorus loading. During the period when CaCO$_3$ was added to the diet the serum phosphorus fell in all groups: PTX 5.0 mg/100 ml; TPTX 4.7 mg/100 ml; CG 5.2 mg/100 ml.

While on the low phosphorus diet without phosphorus supplementation, both the PTX and TPTX groups were more than the control group and had significantly greater phosphorus intake: CG 1300 mg/m$^2$/48 hr; PTX 1820 mg/m$^2$/48 hr ($p<0.01$); TPTX 2120 mg/m$^2$/48 hr ($p<0.001$). There was no significant difference in phosphorus intake between groups during the phosphorus supplementation periods.

While on the low phosphorus diet without phosphate supplementation, the experimental groups (PTX, TPTX) excreted significantly more phosphorus than the control group (CG): CG 305 mg/m$^2$/48 hr; PTX 505 mg/m$^2$/48 hr ($p<0.001$); TPTX 635 mg/m$^2$/48 hr ($p<0.001$) (Fig. 1). There was also a significant difference between the experimental groups ($p<0.01$). There was
no significant difference in the phosphorus excretion between groups in any of the other periods. With cessation of phosphorus loading and the addition of CaCO₃ to the diet all animals dropped their phosphorus excretion to less than 25 mg/m²/48 hr by the second 48-hr collection period.

**DISCUSSION**

It has been adequately shown that PTH is necessary to maintain normal phosphorus balance. Crawford demonstrated that PTX rats given over 1 g per m² of phosphorus/24 hr had a progressive rise in serum phosphorus with an inadequate excretion of phosphorus and diet in tetany. However, our results indicate that rats are able to regulate phosphorus homeostasis independent of changes in PTH secretion. Although, while on a low phosphorus diet the PTX and TPTX animals excreted significantly more phosphorus than the control animals, this was secondary to an increased food intake. All the experimental animals had lost weight after parathyroidectomy, and, with the initiation of PTH replacement, they ate significantly more than the control animals. We have kept PTX rats on this diet and hormone replacement for over a month without a change in serum phosphorus and believe that these rats are not in negative phosphorus balance during this period. The addition of CaCO₃ to bind the phosphorus in the diet produced almost completely
phosphorus-free urine in all groups with a similar drop in serum phosphorus. Thus the PTX and TPTX animals were able to conserve phosphorus normally while on this dose of PTH.

With phosphorus loading, the PTH replaced PTX, and TPTX animals were able to excrete the added phosphorus immediately with no apparent difference from the intact animals. The upper limit of phosphorus tolerance was determined by the development for diarrhea rather than by exceeding the excretory capacity of the kidney. It should be pointed out that, while on 16 mg phosphorus supplement, the animals were receiving greater than 3 g/m²/24 hr of phosphorus, which is much more than any normal diet would contain.

The fall in serum calcium in the experimental groups with the lack of change in the control group indicates that the control group did increase PTH secretion in response to the phosphorus challenge. This is in agreement with other studies of phosphorus loading. The lack of difference in the phosphorus excretion between control and experimental groups either quantitatively or temporally suggests that changes in PTH are not the sole regulator of phosphorus excretion.

The kidney controls the excretion of phosphorus by reabsorption of the phosphorus from the glomerular filtrate. There is no good evidence for tubular secretion of phosphorus. The amount of phosphorus reabsorption is limited (TmP), and, when the filtered phosphorus exceeds TmP, the excess appears in the urine. When filtered phosphorus is less than this limit the urine contains very little phosphorus. PTH is known to decrease TmP. Other factors which affect TmP are volume expansion, glomerular filtration rate, calcitonin, growth hormone, urinary bicarbonate, tubular glucose reabsorption, catecholamines, and magnesium. Whether or not any of these factors have regulatory function in phosphorus metabolism is not known.

There are two possible explanations for the change in phosphorus excretion independent of changes in PTH. One explanation is that phosphorus loading decreases TmP. We have purposely not included calculations of tubular reabsorption of phosphorus (TRP) in our results. Serum phosphorus varies throughout the day. There is no reason to assume that these daily variations are going to be similar on the different diets or that they will be reflected in the morning serum phosphorus determination. We are, therefore, unable to say whether the phosphorus reabsorption changed in our studies.

The rats received 10 mg/100 g body weight more sodium on the 16-mg phosphorus diet than on the low phosphorus diet. This is a significantly increased sodium load. Volume expansion by saline loading has been shown to decrease TmP. This may have contributed to the increased phosphorus excretion. Thyroidectomy did not produce any demonstrable change in phosphorus excretion. Therefore, the change in phosphorus excretion cannot be attributed to calcitonin.

A second explanation for the increased phosphorus excretion is that the TRP may have remained constant, but, with phosphorus loading, the acute rise in serum phosphorus might have been great enough so that the excess phosphorus is excreted, and the serum phosphorus returned to normal within
18 hr after the phosphorus load, the time our serums were obtained. With a creatinine clearance of 35 liter/m²/24 hr, which was the average creatinine clearance of our rats, 14.6 mg/m²/hr of phosphorus would be excreted for each 1 mg/100 ml rise in plasma phosphorus above the level at which TmP is exceeded. This means that plasma phosphorus would have had to have risen a minimum of 6 mg/100 ml to attain the 1000 mg/m²/24 hr, which was excreted during the 16 mg phosphorus diet period. While our experimental design is not capable of differentiating between these possibilities, it presents evidence that PTH is not the only mechanism regulating phosphorus excretion and suggests that other mechanisms are also important.

ACKNOWLEDGMENT

We wish to express our gratitude to Miss Jean Nierman for her technical assistance.

REFERENCES


