# Chronic neutral phosphate supplementation induces sustained, renal metabolic alkalosis

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**Chronic neutral phosphate supplementation induces sustained, renal metabolic alkalosis.** The aim of the present study was to test whether intravenous neutral phosphate supplementation, recently shown in our laboratory to acutely stimulate proton secretion in the distal nephron, was able to induce a sustained metabolic alkalosis. Neutral Na and K phosphate supplementation for seven days, with equivalent reduction in chloride supply and unchanged intake of sodium and potassium, in ADX rats receiving fixed physiological doses of aldosterone and dexamethasone (group 1, $N = 7$), was responsible for a severe metabolic alkalosis (MA; $\Delta$ [$HCO_3$] 11 ± 1.3 mM, and $\Delta$ pH 0.11 ± 0.06 unit). Metabolic alkalosis was at least in part of renal origin, since net acid excretion (NAE) transiently increased, principally due to an increment in titratable acid excretion rate. Balances were equilibrated for sodium and negative for chloride and potassium, which may have contributed to the severity of the MA. Chronic i.v. neutral Na phosphate, without change in potassium and chloride supply, in ADX rats receiving the same doses of steroids (group 2, $N = 5$), was responsible for a less severe MA ($\Delta$ [$HCO_3$] 7.5 ± 0.9 mM, and $\Delta$ pH 0.07 ± 0.01 unit), also of renal origin. In this group, balances were positive for chloride and sodium and equilibrated for potassium. Finally, neutral Na and K phosphate supplementation with reduction in chloride supply in intact rats (group 3, $N = 4$) was also able to induce a MA ($\Delta$ [$HCO_3$] 5.5 ± 1.8 mM, and $\Delta$ pH 0.06 ± 0.01 unit) of renal origin, with balances negative for chloride and equilibrated for potassium and sodium. In all groups, the generation and maintenance of MA probably resulted from stimulated proton secretion in the distal nephron, as suggested by the observed increase of $PCO_2$ over $HCO_3$ concentration ratio in the urine and a fall in urine pH despite augmented urinary buffer content throughout the phosphate infusion period. Glomerular filtration rate did not significantly vary in any group. In conclusion, chronic supplementation of neutral phosphate appears to stimulate *per se* proton secretion in the distal nephron, independently of sodium, chloride, and potassium balances, and adrenal steroid secretion. Thus neutral phosphate supplementation should be added to the previously known factors able to induce MA.

In acute studies, infusion of sodium with neutral phosphate or sulfate to sodium-depleted humans or dogs caused a sharp reduction in urinary pH and an increase in acid excretion [1, 2]. These effects were attributed to the delivery of sodium with a nonreabsorbable anion to the distal nephron that had been stimulated to conserve sodium; it was suggested that the administered anion affects urine pH and acid excretion through its influence on the transepithelial electrical gradient established by active sodium transport across the renal tubular cells [1, 2]. According to this hypothesis, the magnitude of the lumen-negative transepithelial potential difference would be determined by the relative penetrating ability of the available anion. However, chronic infusions of sodium with a nonreabsorbable anion are not expected to result in a sustained increase in net acid excretion [1]. Indeed, the administered sodium is progressively retained by the kidney and the resultant expansion of extracellular fluid volume would suppress the avidity of the distal nephron for sodium [1]. Experimental observations have confirmed this view; chronic infusions of sodium sulfate in dogs fed a low sodium diet or in humans with normal sodium intake did not result in a sustained increase in net acid excretion or increase in plasma bicarbonate concentration [3, 4].

Moreover, among the poorly reabsorbable anion, a specific role for phosphate may be suggested on the basis of other experiments. In our laboratory, we recently showed in the rat that tubular fluid phosphate concentration acutely stimulates proton secretion in collecting tubule independently of its anion and buffer characteristics [5]. This explains the stimulation of proton secretion in the collecting duct observed during acute PTH infusion in TPTX rats [6]. These findings were consistent with previous studies in which an acute infusion of neutral phosphate, but not sulfate, has been shown to enhance urinary net acid excretion in dogs fed a normal sodium intake [7]. Thus tubular fluid phosphate, by its specific stimulating effect on collecting duct proton secretion, may appear as a potent determinant of net acid excretion. These results prompted us to test whether a chronic infusion of neutral phosphate may be responsible for a sustained increase in net acid excretion and plasma bicarbonate concentration, independently of factors already known to induce metabolic alkalosis.

## Methods

Sixteen male Sprague-Dawley rats, weighting 327 ± 3 g, were studied in unrestrained housed metabolic cages. After a seven-day acclimatization period, catheters for chronic infusion and blood samples were implanted under sterile conditions during a light anesthesia (50 mg per 100 g body wt of i.p. pentobarbital). A sterile polyethylene catheter (PE 10) and a sterile silicone polymer catheter (Silastic no. 135, Dow Corning, UK) were implanted into the aorta through the left femoral artery and into the vena cava through the left femoral vein, respectively. Both

catheters were exteriorized at the back of the neck through a stainless steel ring sewed on the skin and then inserted into a 30 cm-long stainless steel protective spring. The inferior end of this spring was bound up to the ring and the superior one was bound up to a swivel canulla (Instech Laboratories, Horsham, USA) which allowed animal to freely move in the cage without any risk of twisting the catheters. The arterial catheter was filled with a sterile polyvinylpyrolidone solution (Prolabo, France) and its extremity was heat sealed. The venous catheter was connected to the pump through the canulla and continuous infusion of adequate solution was started. Some rats were surgically adrenalectomized (ADX), through a bilateral posterior incision, just after the implantation of catheters, and then received continuous infusion of physiological replacement doses of aldosterone (0.5 $\mu$g/100 g body wt/day) and dexamethasone (1.2 $\mu$g/100 g body wt/day) throughout the study [8], whereas other rats were sham operated.

Blood samples were obtained from the arterial catheter, previously emptied of polyvinylpyrolidone. Each blood sample was immediately followed by a low rate transfusion (1 ml per 5 min) of an adequate amount of whole blood obtained on the morning of the study from another Sprague-Dawley rat that was maintained on the same diet as the experimental rat, and either ADX the day before or intact as appropriate. Urine was daily collected throughout the study, in graduates containing water saturated mineral oil and thymol crystals. At the end of each seven-day period, glomerular filtration rate (GFR) was estimated from inulin clearance (priming i.v. dose of 20 $\mu$Ci of [Methoxy-$^3$H] inulin; New England Nuclear, Boston, Massachusetts, USA; and sustained infusion of 5 $\mu$Ci/hr). After a 90-minute equilibration period, a four hour urine collection was begun and three blood samples were drawn during this period.

### Protocols

Animals had free access to distilled water and were given daily a fixed amount (20 g) of a regular rat chow that they completely ate (AO4, UAR, France). Mineral contents were in g/kg of food: sodium, 2.4; chloride, 2.5; potassium, 6; calcium, 6; phosphorus, 5.9. The rate of continuous intravenous infusion of adequate solutions was 1 ml/hr.

The study was divided into three successive, seven day periods: control, neutral phosphate infusion and recovery. The first period started 48 hours after surgery. Animals were divided into 3 groups.

*Group 1* (N = 7, 325 ± 6 g).Intravenous chronic neutral phosphate supplementation with equivalent reduction in chloride and unchanged infusion rates of sodium and potassium in ADX rats receiving fixed doses of steroids. Infusate compositions were (mM): NaCl, 115.4 and KCl, 167.5 during control and recovery periods; NaH$_2$PO$_4$ 10.63, Na$_2$HPO$_4$, 53.13, K$_2$HPO$_4$, 74.7 and KH$_2$PO$_4$, 17.9 during phosphate period.

*Group 2* (N = 5, 324 ± 4 g).Intravenous chronic neutral phosphate supplementation without change in chloride supply in ADX rats receiving the same daily fixed doses of steroids as in Group 1. Infusion rates were unchanged for potassium but increased for sodium. In addition, to avoid occurrence of relative extracellular fluid (ECF) volume contraction during recovery period, infused amount of sodium was held constant over the last two periods. Infusate compositions were (mM): NaCl, 115.4 and KCl, 167.5 during control period; NaCl, 115.4,

KCl, 167.5, Na$_2$HPO$_4$, 131.6 and NaH$_2$HPO$_4$, 26.3 during phosphate period; and NaCl, 404.9 and KCl, 167.5 during recovery.

*Group 3* (N = 4, 336 ± 5 g).Infusate compositions were the same as in Group 1 for the three periods, but the rats were not ADX (sham-operated) and therefore able to modulate the endogenous secretion of steroids.

### Analytical procedures and calculations

[Methoxy-$^3$H] inulin activity in plasma and urine was measured by scintillation counting (LKB, Bromma, Sweden). Urine and plasma sodium and potassium concentrations were measured by indirect potentiometry (Model E2A, Beckman Instruments, Fullerton, California, USA); urine and plasma chloride concentrations by a chloride titrator (Model CMT10, Radiometer, Copenhagen, Denmark); urine and plasma phosphorus concentrations by colorimetric determination. Plasma protein concentration was determined by refractometry; pH and PCO$_2$ in plasma and urine by a gas analyzor (Radiometer), total CO$_2$ in urine by thermic conductivity (Model 965, Corning); titratable acid and ammonium urinary concentrations were determined by titration methods [9, 10]. Titratable acid attributable to phosphate was calculated using the Henderson-Hasselbalch equation and a pK of 6.8. Apparent bicarbonate space (A.B.S.) was estimated for each group of animals, using an algorithm developed by Fernandez, Cohen and Feldman [11]:

$$A.B.S. = \left[0.4 + \frac{2.6}{[HCO_3]p}\right] \times body\ weight$$

in which [HCO$_3$⁻]p is plasma bicarbonate concentration.

Statistical analysis has been performed by one-way and two-way analysis of variance, Scheffé, nonparametric Wilcoxon, and paired $t$-test as appropriate. Results are expressed as mean ± SEM. $P < 0.05$ was considered significant.

### Results

*Effects of chronic supplementation of neutral phosphate with reduction in chloride supply and unchanged intake of sodium and potassium, in ADX rats receiving fixed physiological replacement doses of steroids (Group 1)*

The design of this group was to investigate the effects of chronic neutral phosphate infusion while other factors known to influence distal proton secretion (that is, plasma mineralocorticoid concentration, sodium and potassium supply) were controlled.

*Acid-base changes.* Plasma bicarbonate concentration increased by the second day of phosphate administration and remained elevated throughout this period. The difference in bicarbonate concentration reached 11 ± 1.3 mM. Then plasma bicarbonate concentration promptly fell to normal during the recovery period. Plasma pH also increased (0.11 unit ± 0.06) during phosphate infusion while PaCO$_2$ did not vary significantly (Fig. 1).

The time course of urine acid-base parameters is summarized in Figure 2. Compared to control period, urinary pH was decreased throughout the phosphate infusion period (7.05 ± 0.01 vs. 7.24 ± 0.05, $P < 0.05$) and then returned to values not
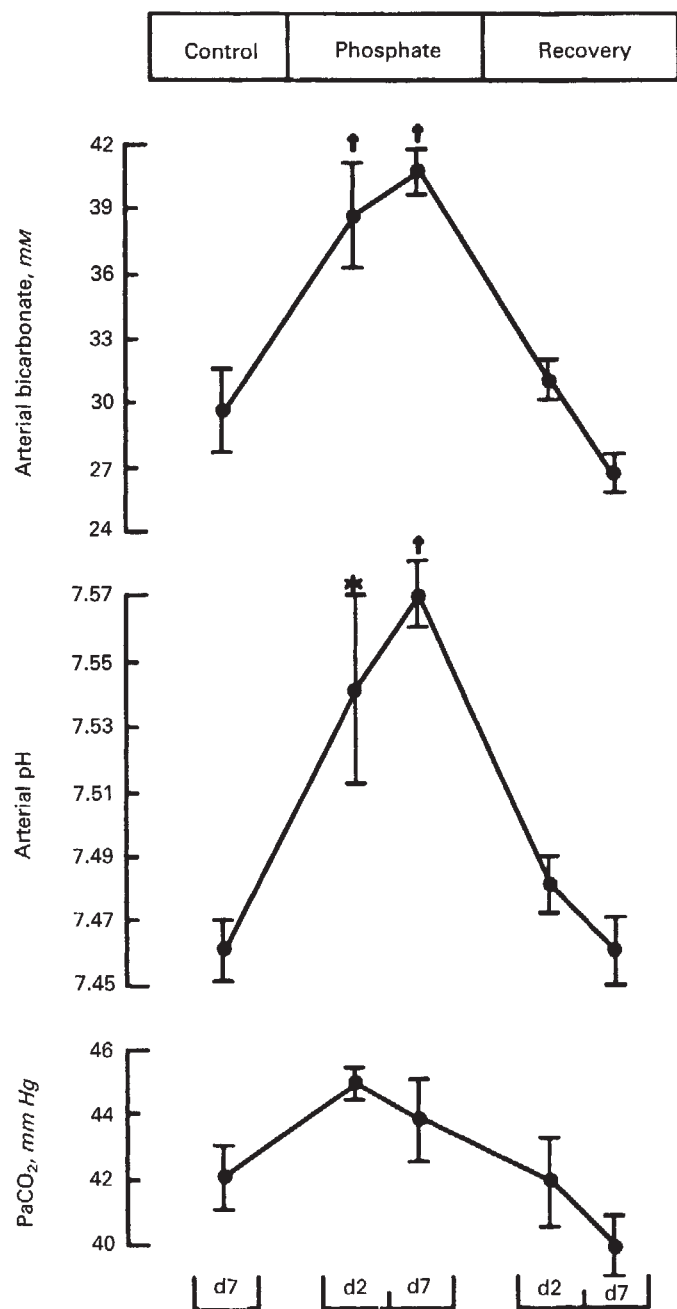
**Fig. 1.** *Effects of chronic supplementation of neutral phosphate and chloride restriction on arterial acid-base composition in ADX rats receiving a fixed Na and K supply and physiological replacement doses of steroids (group 1).* Values were tested for significant differences from control: * $P < 0.01$; $\blacktriangle P < 0.001$.

different from control during recovery (7.36 ± 0.03). Mean daily urinary net acid excretion (NAE), expressed as the difference from end-control period values, significantly increased on the first day of phosphate infusion (increment of 0.55 ± 0.14 mmol/24 hr, $P < 0.05$) and then progressively decreased to values not different from controls during the last four days of this period. During the recovery period NAE transiently decreased to values lower than controls as expected (Fig. 2). The

cumulative negative balance of proton calculated during phosphate infusion period (−1.87 ± 1.61 mmol) was not significantly different from the calculated cumulative retention of proton during recovery (2.59 ± 1.22 mmol). The corresponding addition of bicarbonate (1.87 mmol) to an estimated A.B.S. of 159 ml (according to the animals' mean weight and control plasma bicarbonate concentration) was expected to induce an increase in plasma bicarbonate concentration of 11.8 mM, a value very close to that actually observed (see above). Mean daily urinary titratable acid excretion was increased throughout the phosphate infusion period (0.37 ± 0.04 vs. 0.01 ± 0.02 mmol/24 hr, $P < 0.01$) and then promptly returned to values similar to controls during recovery (0.01 ± 0.01 mmol/24 hr). Calculated titratable acid excretion attributable to phosphate was 0.01 ± 0.06, 0.27 ± 0.15, and 0.00 ± 0.07 mmol/24 hr during the control, phosphate, and recovery periods, respectively, which was thus consistent with measured titratable acid excretion. Mean daily ammonium excretion remained unchanged throughout the study (0.54 ± 0.03, 0.54 ± 0.06, and 0.55 ± 0.09, in control, experimental, and recovery periods, respectively). Mean daily urinary bicarbonate excretion decreased on the first day of phosphate infusion period (0.97 ± 0.08 vs. 1.19 ± 0.06 mmol/24 hr), although not significantly, and then increased to values above controls during the last 6 days of the experimental period (1.30 ± 0.07 vs. 1.19 ± 0.06 mmol/24 hr, $P < 0.005$). During recovery period, mean daily bicarbonate excretion increased further (1.56 ± 0.07 mmol/24 hr, $P < 0.03$), which accounted for the decrease in NAE.

The urinary $PCO_2$ over bicarbonate concentration ratio, used as an index of the collecting duct proton secretion rate [12], was significantly higher throughout the phosphate infusion period than the control value (4.10 ± 0.28 vs. 2.06 ± 0.36, $P < 0.05$). In addition, a positive linear correlation between the latter index and urinary phosphate concentration was found when the data of the three periods were pooled (Fig. 3).

*Miscellaneous.* Plasma sodium and phosphate concentrations remained unchanged, whereas potassium and chloride concentrations decreased significantly during phosphate infusion period and returned to normal during recovery period (Table 1).

As expected, mean urinary phosphate excretion dramatically increased during phosphate infusion (3.23 ± 0.19 vs. 0.20 ± 0.04 mmol/24 hr) as well as urinary phosphate concentration (104.7 ± 6.7 vs. 7.9 ± 1.1 mM). Both values returned to control levels during recovery.

Sodium, potassium, and chloride balances were estimated as the differences between intakes (intravenous and dietary) and urinary excretion rates (Table 2). During control period, sodium and chloride balances were equilibrated. Sodium balance was equilibrated over the three periods; indeed, plasma protein concentration, hematocrit, and GFR did not change over the entire study. A negative cumulative balance for chloride developed as expected during phosphate period (−2.77 ± 0.69 mmol, $P < 0.01$) and repaired during recovery (2.99 ± 1.23 mmol, $P < 0.01$). With respect to potassium balance, the discrepancy between intake and urinary excretion rate during control period may be attributed to potassium retention for normal growth, as previously observed by others [13, 14]. Thus possible changes in the potassium balance between periods were calculated from the differences in urinary excretions. A negative cumulative balance for potassium (−3.5 ± 0.7 mmol, $P < 0.001$) also
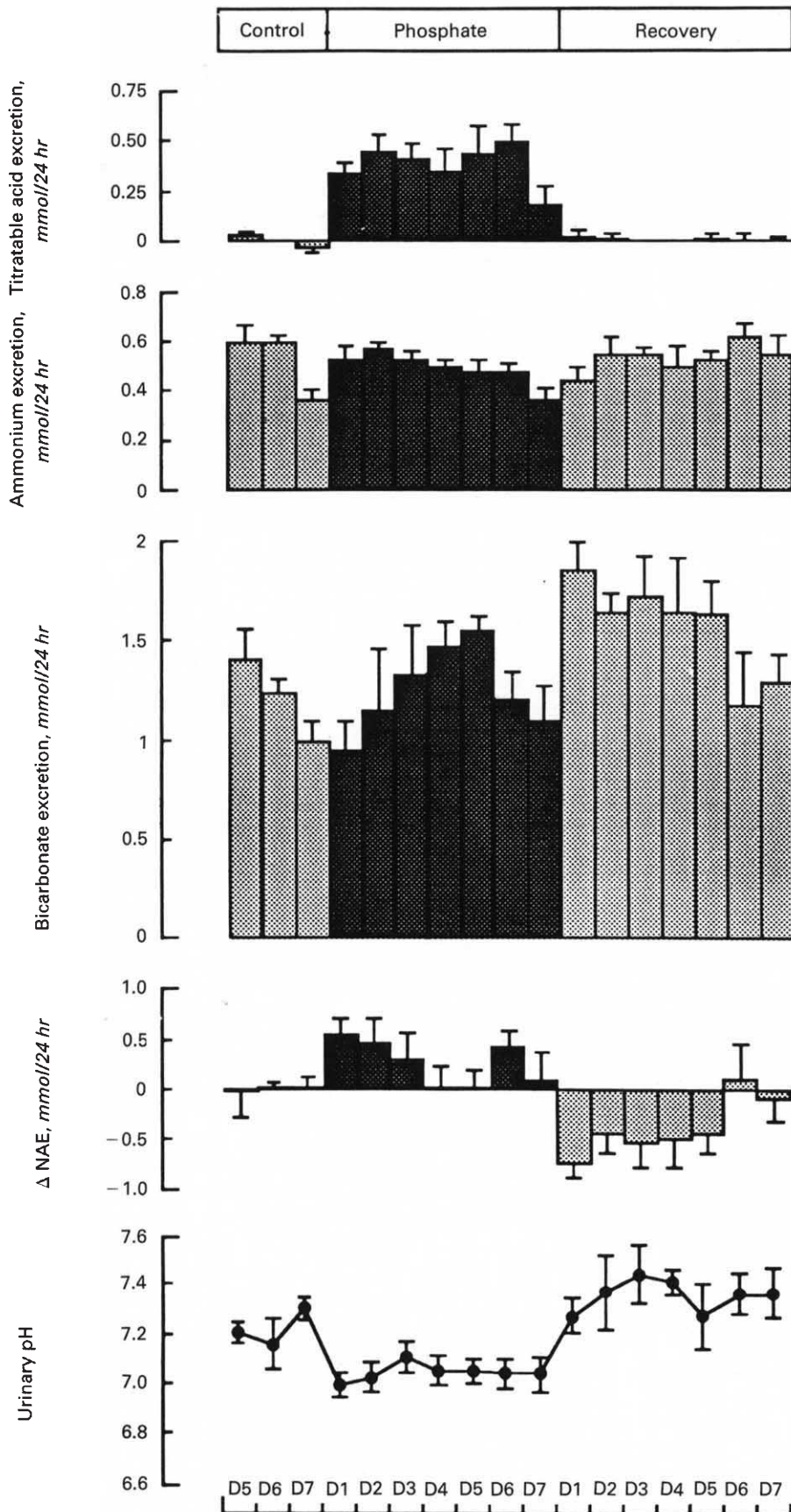
**Fig. 2.** *Time course of urinary acid-base composition in rats of group 1.* Δ NAE: mean daily net acid excretion rates expressed as the difference to the mean end-control period value.
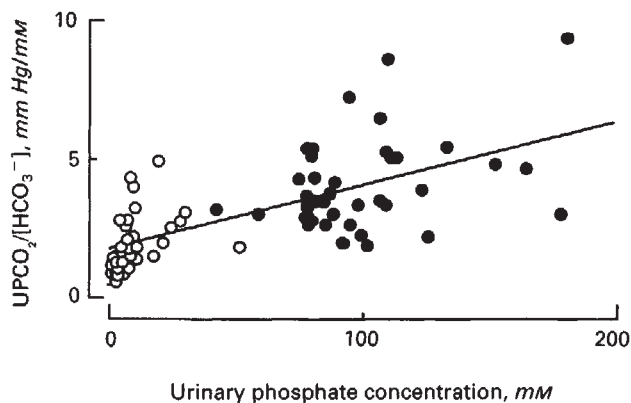
**Fig. 3.** *Relationship between urinary $PCO_2$ over urinary bicarbonate concentration ratio and urinary phosphate concentration in rats of group 1.* Neutral phosphate infusion period (○); control and recovery periods (●). $N = 91$; $r = 0.61$; $P < 0.001$; $y = 1.80 + 0.023x$.

developed during phosphate period and remained unchanged during recovery.

Thus, under conditions of fixed plasma gluco- and mineralocorticoid concentrations and sodium balance equilibrium, a frank metabolic alkalosis of renal origin occurred during neutral phosphate infusion. However, negative balances for chloride and potassium may have contributed to the severity of metabolic alkalosis.

*Effects of chronic supplementation of neutral phosphate and sodium, without change in potassium and chloride intake, in ADX rats receiving fixed physiological replacement doses of steroids (Group 2)*

This group was designed to investigate the effects of chronic neutral phosphate infusion in the absence of negative chloride and potassium balance.

Plasma and urine acid-base composition is shown in Figure 4. Metabolic alkalosis also occurred during phosphate administration, but the elevations in plasma bicarbonate concentration ($\Delta$ [$HCO_3$] $7.5 \pm 0.9$ mM, $P < 0.01$) and arterial pH ($\Delta$ pH $0.07 \pm 0.01$ unit, $P < 0.001$) at the seventh day of phosphate infusion were less important than in group 1; both values returned to near normal during recovery. Variations in urinary pH and NAE during phosphate infusion were in the same direction as in group 1, but less important. The cumulative negative proton balance ($-1.46 \pm 0.57$ mmol, $P < 0.05$) during phosphate infusion was followed by a statistically similar cumulative retention of proton during recovery ($2.44 \pm 0.69$ mmol). Into an estimated A.B.S. of 159 ml, such an addition of bicarbonate (1.46 mmol) was expected to induce an increase in plasma bicarbonate concentration of 9.2 mM, which was consistent with the observed value. As in group 1, mean daily titratable acid excretion increased throughout phosphate infusion period ($0.41 \pm 0.01$ vs. $0.03 \pm 0.01$ mmol/24 hr, $P < 0.001$) while ammonium excretion remained constant ($0.60 \pm 0.03$ vs. $0.61 \pm 0.03$ mmol/24 hr). Titratable acid excretion attributable to phosphate was $0.00 \pm 0.05$ and $0.27 \pm 0.14$ mmol/24 hr during the control and phosphate periods, respectively. Mean bicarbonate excretion slightly increased ($1.67 \pm 0.08$ vs. $1.48 \pm 0.14$ mmol/24 hr) mainly by the fourth day of the phosphate infusion

period, and thereby allowed NAE to return to near control values. As in group 1, urinary $PCO_2$ rose during phosphate infusion ($124 \pm 6$ vs. $77 \pm 9$ mm Hg, $P < 0.001$) and corrected during the third period ($63 \pm 9$ mm Hg).

Sodium, chloride and potassium cumulative balances are depicted in Figure 4. A positive cumulative sodium balance developed during phosphate administration ($4.9 \pm 4.9$ mmol) and sustained after discontinuation of phosphate infusion. Plasma sodium concentration did not change. Plasma chloride concentration decreased during phosphate infusion ($98.0 \pm 0.9$ vs. $104.0 \pm 1.5$ mM, $P < 0.01$). Urinary chloride excretion remained unchanged ($8.72 \pm 0.35$ vs. $8.59 \pm 0.34$ mmol/24 hr) so that chloride balance was equilibrated; a positive cumulative chloride balance ($7.00 \pm 1.02$ mmol; Fig. 4) developed during recovery, according to the enhanced chloride supply. Thus, as expected, chloride depletion did not occur in this group. Plasma potassium concentration decreased during phosphate infusion ($3.34 \pm 0.26$ vs. $4.30 \pm 0.24$ mM, $P < 0.001$), and remained so during recovery ($3.55 \pm 0.11$ mM). Daily urinary potassium excretion did not change during phosphate infusion ($6.87 \pm 0.18$ vs. $6.75 \pm 0.21$ mmol/24 hr) and during the third period ($6.63 \pm 0.15$ mmol/24 hr). Therefore, the potassium balance remained equilibrated throughout the study. GFR values tended to increase during phosphate infusion ($2.59 \pm 0.18$ vs. $2.27 \pm 0.13$ ml/min) and recovery ($2.77 \pm 0.11$ ml/min), although not significantly.

In conclusion, under conditions of fixed plasma gluco- and mineralocorticoid concentrations, potassium and chloride balance equilibrium, and positive sodium balance, a metabolic alkalosis of renal origin occurred during neutral phosphate infusion.

*Effect of chronic supplementation of neutral phosphate with reduction in chloride intake, and unchanged intake of sodium and potassium, in intact rats (Group 3)*

This group was designed to determine the effects of prolonged neutral phosphate administration in animals with controlled sodium and potassium intake but preserved ability to regulate endogenous adrenal steroids secretion.

The main results are shown in Figure 5. The elevations in plasma bicarbonate ($\Delta$ [$HCO_3$] $5.5 \pm 1.8$ mM, $P < 0.05$) and pH ($\Delta$ pH $0.06 \pm 0.01$ unit, $P < 0.05$) at the seventh day of phosphate infusion period were slightly less than those found in group 2. Then these values returned to control during recovery. Compared to the control period, urinary pH was decreased all over the phosphate infusion ($7.16 \pm 0.03$ vs. $7.40 \pm 0.10$, $P < 0.01$) and returned close to control values during recovery ($7.39 \pm 0.06$). The negative cumulative proton balance ($-1.19 \pm 0.94$ mmol) during phosphate infusion was followed by a similar retention of proton during recovery ($2.2 \pm 0.95$ mmol). For an assumed A.B.S. of 169 ml, according to the animals' mean weight and control plasma bicarbonate concentration, such an amount of added bicarbonate (1.19 mmol) would induce an increase in plasma bicarbonate concentration of 7.04 mM consistent with the observed value.

The increase in titratable acid excretion ($0.22 \pm 0.03$ vs. $0.00 \pm 0.01$ mmol/24 hr, $P < 0.001$) over the whole period mainly accounted for changes in NAE excretion while ammonium excretion remained constant ($0.42 \pm 0.02$ vs. $0.43 \pm 0.06$

**Table 1.** Effect of chronic infusion of neutral phosphate on blood variables and glomerular filtration rate in rats of group 1 ($N = 7$)

| Period | Na | K | Cl | Pi | Protein | Hct | GFR |
|---|---|---|---|---|---|---|---|
| | mM | | | | g/liter | % | ml/min |
| Control | | | | | | | |
| Day 7 | 145.0 ± 1.4 | 4.40 ± 0.02 | 104.6 ± 1.2 | 2.18 ± 0.09 | 49.3 ± 1.9 | 44.6 ± 1.0 | 2.87 ± 0.04 |
| Phosphate | | | | | | | |
| Day 7 | 143.4 ± 1.1 | 3.38 ± 0.15[a] | 92.7 ± 1.0[b] | 2.24 ± 0.10 | 53.7 ± 2.7 | 44.0 ± 0.8 | 2.90 ± 0.01 |
| Recovery | | | | | | | |
| Day 7 | 143.4 ± 1.1 | 4.31 ± 0.19 | 105.6 ± 2.0 | 2.01 ± 0.16 | 53.0 ± 1.9 | 44.6 ± 2.5 | 2.57 ± 0.01 |

Abbreviations are: Hct, hematocrit; GFR, glomerular filtration rate. Values are means ± SEM. Mean values were tested for significant differences from control: [a] ($P < 0.01$), [b] ($P < 0.001$)

**Table 2.** Mean daily intakes (dietary and intravenous), urinary excretion rates for Na, K, Cl, and urinary Cl concentration in rats of group 1 ($N = 7$)

| Periods | Daily balance | Na | K | Cl | Cl |
|---|---|---|---|---|---|
| | | mmol/24 hr | | | mM |
| Control | Intake | 4.38 | 7.02 | 8.19 | |
| | Urinary excretion | 4.14 ± 0.21 | 6.49 ± 0.2 | 8.13 ± 0.28 | 305 ± 25 |
| Phosphate | Intake | 4.38 | 7.02 | 1.40 | |
| | Urinary excretion | 4.18 ± 0.11 | 6.99 ± 0.17 | 2.38 ± 0.33 | 81 ± 7 |
| Recovery | Intake | 4.38 | 7.02 | 8.19 | |
| | Urinary excretion | 4.12 ± 0.11 | 6.58 ± 0.13 | 7.60 ± 0.45 | 246 ± 30 |

mmol/24 hr). Titratable acid excretion attributable to phosphate was 0.00 ± 0.06 and 0.16 ± 0.12 mmol/24 hr during the control and phosphate periods, respectively. Bicarbonate excretion increased during phosphate period, mainly by the fifth day (1.39 ± 0.17 vs. 1.17 ± 0.21 mmol/24 hr), allowing NAE excretion to return toward control values. Urinary $PCO_2$ rose during phosphate infusion (115 ± 5 vs. 78 ± 16 mm Hg, $P < 0.05$), and corrected during the third period (81 ± 10 mm Hg). Because of a slight decrease in urinary sodium excretion during phosphate infusion (3.56 ± 0.13 vs. 3.77 ± 0.25 mmol/24 hr), cumulative sodium balance tended to be positive, although not significantly. A negative cumulative chloride balance (−3.54 ± 1.40 mmol) developed during phosphate infusion which corrected when chloride was reintroduced. Plasma potassium concentration remained unchanged during phosphate infusion (4.4 ± 0.2 vs. 4.7 ± 0.2 mM) and urinary potassium excretion tended to decrease with a slight positive balance (0.69 ± 1.25 mmol). Finally, GFR values remained constant during phosphate infusion (2.75 ± 0.23 vs. 2.62 ± 0.33 ml/min), as did plasma protein concentration and hematocrit.

### Discussion

This study demonstrates that a sustained metabolic alkalosis of renal origin develops during chronic neutral phosphate supplementation: (1) in ADX rats with fixed physiological steroid replacement, equilibrated sodium balance and potassium and chloride depletion (group 1); (2) in ADX rats with fixed physiological steroid replacement, equilibrated chloride and potassium balance and ECF volume expansion (group 2); and (3) in intact rats with equilibrated sodium and potassium balance but chloride depletion (group 3). Taken together these findings demonstrate that phosphate administration was the main factor that caused metabolic alkalosis in this study.

Metabolic alkalosis was generated by renal mechanisms since net acid excretion increased while alkalosis was developing. In each group of this study, the severity of metabolic alkalosis was fully explained by the positive bicarbonate balance generated by the kidney. Provided that the calculation of the A.B.S. is valid for chronic metabolic alkalosis, no net hydrogen ion shift between extra- and intracellular spaces occurred in our study. The increase in NAE was mainly the consequence of enhanced phosphaturia and lowered urinary pH since the enhancement in titratable acidity attributable to phosphate accounted for 70 to 73% of the total increase in titratable acid excretion. The stimulation of tubular proton secretion probably took place in the distal nephron, as indicated by the increase in $PCO_2$ over bicarbonate concentration ratio in the urine, which occurred as early as the first day of phosphate infusion. The stimulation of collecting duct proton secretion was also attested by the decrease in the urinary pH despite an increase in the urinary buffer content during phosphate infusion. This is in agreement with the stimulatory effect of phosphate on distal proton secretion reported in acute studies [5].

The results of this study indicate that renal mechanisms also accounted for the maintenance of metabolic alkalosis induced by phosphate infusion. Indeed, after metabolic alkalosis had fully developed and a new steady state was reached, net acid excretion returned to a stable rate not different from the rate observed prior to phosphate infusion.

Two main mechanisms have been implicated in the maintenance of metabolic alkalosis by the kidneys: first, a decrease in GFR which prevents an increase in the filtered load of bicarbonate [15]; second, an increase in bicarbonate tubular reabsorption [16]. In the present study, the main role seems to be devoted to increased tubular reabsorption since GFR remained stable in groups 1 and 3 and even tended to increase in group 2 during neutral phosphate infusion, so that the bicarbonate filtered load increased during phosphate infusion in the three groups. Indeed, the fractional excretion of bicarbonate (calculated on the last day of control and phosphate periods) remained
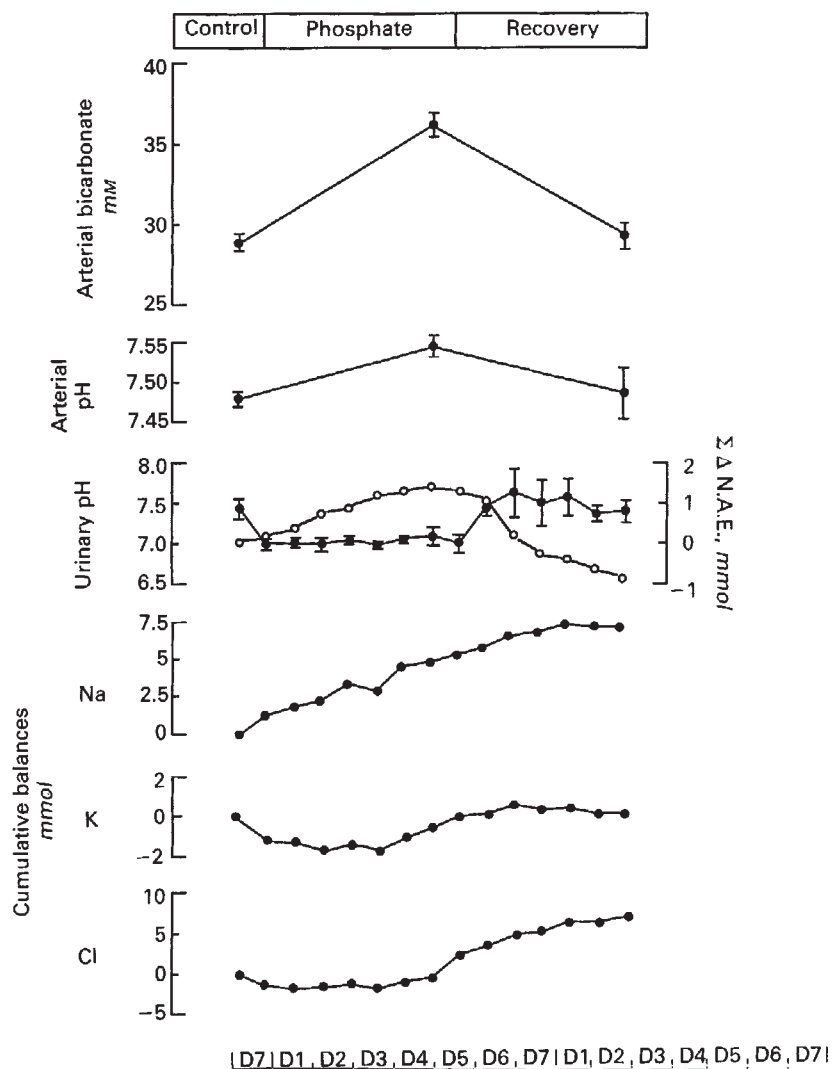
**Fig. 4.** *Effects of chronic supplementation of neutral phosphate and Na on arterial acid-base composition, urinary pH (●), cumulative variations in NAE (○; a positive value indicates increased excretion), and cumulative balances for Na, K, Cl, in ADX rats receiving a fixed Cl and K supply and physiological replacement doses of steroids (group 2).*

constant in each group, indicating that an increase in absolute tubular reabsorption of bicarbonate occurred during phosphate infusion. The distal nephron probably contributed at least in part to the increased tubular reabsorption of bicarbonate. Indeed, the abnormally high $PCO_2$ over bicarbonate concentration ratio in urine throughout the phosphate infusion period [12] and the persistent decrease in urinary pH concomitant with an increase in urinary phosphate concentration indicate a sustained increase in distal proton secretion. This situation differs strikingly from the renal response to chronic dietary bicarbonate challenge in normal rat. In the latter situation, the distal tubular reabsorption of bicarbonate is adequately lowered, which prevents the occurrence of metabolic alkalosis [17].

Taken together, these results strongly support that the distal nephron had a major role in both the generation and maintenance of metabolic alkalosis. The lack of decrease of ammonium excretion during metabolic alkalosis is surprising, since metabolic alkalosis is known to depress ammonia synthesis in proximal tubule [18]. Two mechanisms may have counterbalanced the latter effect: first, in all groups of rats, the observed lowering in urinary pH, which decreases the urinary $NH_3$

concentration by shifting the buffer equilibrium away from $NH_3$ thereby enhancing the peritubular-to-lumen $NH_3$ gradient [19], has been shown to enhance ammonium excretion [20]. Second, hypokalemia observed in groups 1 and 2, has been reported to stimulate ammonia synthesis [21].

The occurrence of metabolic alkalosis of renal origin during phosphate administration in the present study leads us to examine the different physiological mechanisms known to induce this acid-base disturbance, such as mineralocorticoid excess, ECF volume contraction, and chloride and potassium depletion.

Mineralocorticoids are known to directly stimulate proton secretion in the collecting duct, independently of their action on ECF volume and plasma potassium concentration [22]. This factor cannot be invoked *per se* in the present study since animals of groups 1 and 2 had physiological steroid replacement. However, it could be argued that a rather high sodium supply together with a fixed aldosterone replacement might have resulted in a state of relative mineralocorticoid excess and attendant permanent stimulation of distal proton secretion; in this case, phosphate administration, resulting in abundant distal
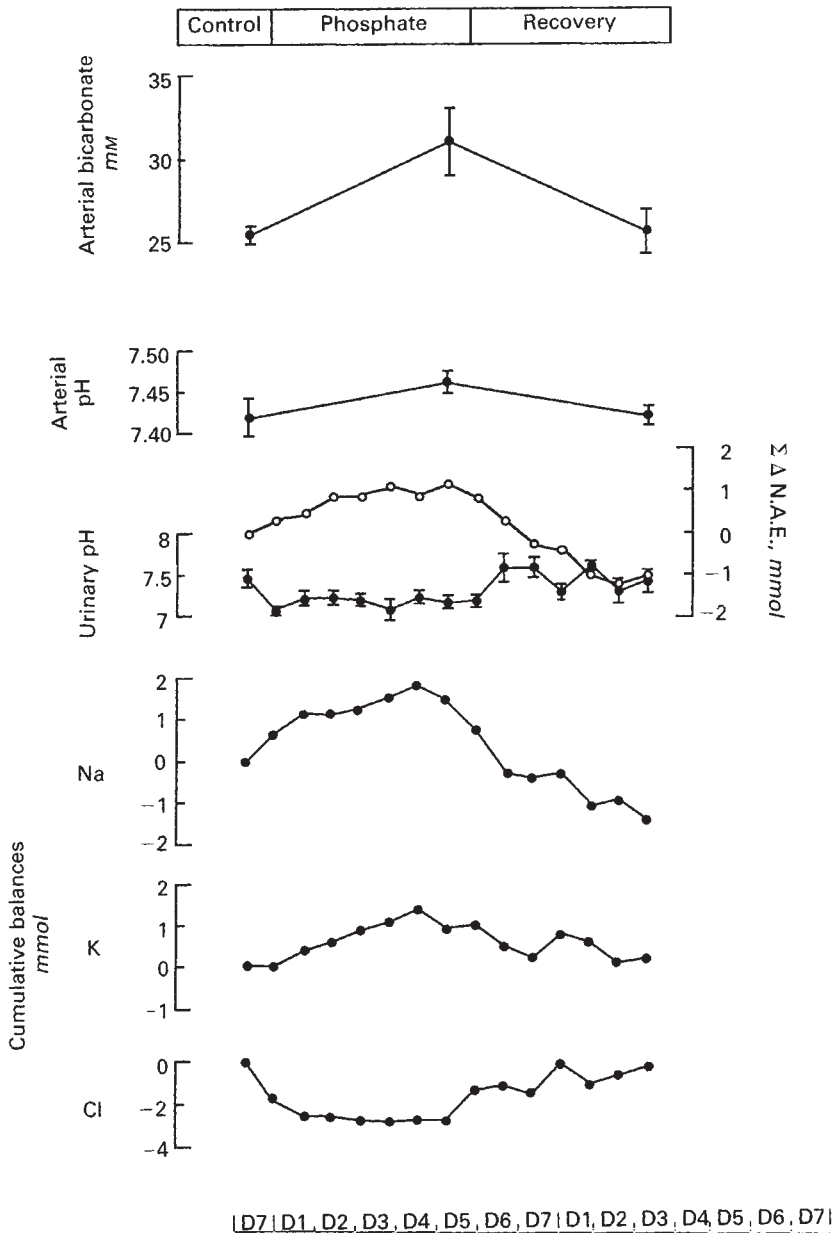
**Fig. 5.** *Effects of chronic neutral phosphate supplementation and chloride restriction on arterial acid-base composition, urinary pH (●), cumulative variations in NAE (○; a positive value indicates increased excretion), and cumulative balances for Na, K, Cl, in intact rats receiving a fixed Na and K supply (group 3).*

buffer delivery, would increase titratable acid and thus net acid excretion without any further decrease in urinary pH. Indeed, the urinary pH should have, if anything, increased during phosphate infusion because the high urinary buffer content should have limited urinary acidification by collecting duct proton secretion. By contrast, in the present study urinary pH steadily decreased in groups 1 and 2 during phosphate administration, suggesting that phosphate *per se* stimulated distal proton secretion. Nevertheless it cannot be excluded that a relative mineralocorticoid excess could have contributed to the severity of the metabolic alkalosis observed in groups 1 and 2. In addition, such a relative aldosterone excess cannot be invoked in group 3 in which animals had an intact ability to regulate endogenous aldosterone secretion that should have been depressed by the high sodium supply.

ECF volume contraction has been shown to generate [23, 24] and maintain [16] metabolic alkalosis by stimulating tubular reabsorption of bicarbonate. In the present study, this factor cannot be invoked since sodium balance was either equilibrated (groups 1 and 3) or positive (group 2).

Chloride depletion has also been proposed to be a major determinant in the maintenance of metabolic alkalosis [25, 26]. In groups 1 and 3, we cannot exclude that the observed chloride depletion contributed to the maintenance of metabolic alkalosis. However, a negative chloride balance is not a prerequisite since in group 2, in which metabolic alkalosis also developed, a positive chloride balance occurred (Fig. 4). In addition, urinary chloride concentration was sufficiently high during phosphate infusion in the three groups (≥80 mM) to suggest that phosphate-induced metabolic alkalosis was chloride-resistant.

Selective dietary potassium depletion has been shown in rats to generate metabolic alkalosis without variation in proton balance [27–29]. Then the alkalosis is maintained by a decrease in GFR and/or an increase in tubular reabsorption of bicarbonate. It is possible that the potassium depletion in group 1 contributed to the severity of the metabolic alkalosis. However, a negative potassium balance is not necessary for metabolic alkalosis to occur during phosphate infusion, since in groups 2 and 3 the potassium balance remained equilibrated (Fig. 5).

Since metabolic alkalosis has developed independently of variations in Na intake and ECF volume, chloride and potassium balance, and adrenal steroid secretion, chronic neutral phosphate infusion appears to stimulate *per se* distal tubular proton secretion sufficiently to generate and sustain a metabolic alkalosis.

The present results extend to chronic situations those previously obtained from acute studies in our laboratory. We have recently stressed in acute studies that phosphate is a potent stimulus for acidification in the collecting duct, and that the degree of stimulation is positively correlated to the urinary phosphate concentration [5]. The mechanism remains uncertain; some degree of inhibition of the luminal $Cl^-/HCO_3^-$ exchange responsible for bicarbonate secretion by the $\beta$ intercalated cells might be considered since, in groups 1 and 3, the complete replacement of chloride by neutral phosphate in perfusate (but not in the chow) lowered the urinary chloride concentration in the final urine from # 300 to # 80 mM. However metabolic alkalosis occurred in group 2, in which a high urinary chloride concentration (>100 mM) was maintained during phosphate infusion. Therefore, a direct stimulation by phosphate of proton secretion by alpha intercalated cells seems much more likely. As discussed in the introduction, the poor reabsorbability of the anion phosphate is not sufficient to account for a sustained increase in tubular proton secretion and thus metabolic alkalosis. Indeed, chronic administration of another poorly reabsorbable anion, sulfate, is not accompanied by metabolic alkalosis [3, 4]. Urinary phosphate has also been suggested to act via its buffer characteristics [7], but the latter properties cannot account for the observed fall in urinary pH. The capacity of phosphate to strongly stimulate distal proton secretion may be due to additional mechanisms, as discussed in a previous paper from our laboratory [5]. Thus, it can be proposed that, by combining these properties, high urinary phosphate concentration accounted for a sustained increase in distal tubular proton secretion that generated and maintained metabolic alkalosis for up to seven days. This potent effect of urinary phosphate could explain at least in part the generation of metabolic alkalosis observed in experimentally-induced hyperparathyroid states in humans and animals, in which PTH-induced phosphaturia persist for several days [reviewed in 5].

More generally, the role of neutral phosphate supply in the pathogenesis of experimental metabolic alkalosis ought to be reappraised, particularly in some situations in which chloride depletion has been suggested to play a predominant role. For example, in the model of chloride-depletion alkalosis generated in the rat by peritoneal dialysis with sodium and potassium bicarbonate solutions [25], the chloride-free diet necessary to maintain chloride depletion alkalosis without bicarbonate administration contained neutral sodium phosphate, as pointed out previously [30]. In other experiments, recovery from met-

abolic alkalosis induced by gastric drainage in animals maintained on a low-sodium and low-chloride diet was prevented by a sodium neutral phosphate supply whereas animals receiving sodium chloride corrected alkalosis within 48 hours [31]. In metabolic alkalosis produced in humans by administration of furosemide and maintained by restriction of sodium chloride intake, an amount of sodium phosphate intake sufficient to correct the negative sodium balance worsened the metabolic alkalosis by increasing renal bicarbonate generation [26]. Taken together, these observations raise the possibility of a role for neutral phosphate administration, besides chloride depletion, in the pathogenesis of metabolic alkalosis. Thus, neutral phosphate supplementation should be added to the previously known factors able to induce metabolic alkalosis.

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