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Dietary phosphate-dependent growth is not mediated by changes in plasma phosphate concentration

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The present study was undertaken in order to assess the role of dietary phosphate in growth. A diet deficient in phosphate led to a suppression of growth in juvenile rats. The phenomenon is similar to that described for diets deficient in other essential single components such as Mg, Zn or K. However, unlike the other constituents, dietary phosphate restriction affected the growth rate much more than it altered the serum phosphate concentration; addition of phosphate to the drinking water of rats fed a low-phosphate diet restored the growth rate without a concomitant change in serum phosphate concentration. The suppression of growth rate by the deletion of phosphate was associated with a delayed decrease in food intake. This finding implies that the variation in appetite was secondary to the change in growth. The increase in body weight following phosphate supplementation was associated with a concomitant increment in food intake. The phosphate-dependent growth was, however, evident also in rats that were pair-fed with those that were not supplied with phosphate. It is concluded that dietary phosphate-dependent growth is not mediated by changes in phosphate concentrations in the extracellular fluid. It is plausible that signals arising from receptors for phosphate in the digestive system constitute part of the growth control apparatus in rats.

Dietary phosphate: Low phosphate diet: Growth: Intestinal signals: Appetite: Food intake

Nutritional signals from the digestive system play a significant role in the metabolic homeostatic mechanism of the organism. The best known example of these intestinal signals is the incretin phenomenon in glucose homeostasis (McIntyre *et al.* 1965; Creutzfeldt, 1980). The main feature of the incretin phenomenon is that glucose applied through the digestive system is much more effective in stimulating insulin release than glucose given intravenously (McIntyre *et al.* 1965). It is now evident that insulinotropic hormones such as glucagon-like peptide I and gastric inhibitory peptide are involved. These hormones are released by the intestinal epithelium in response to the glucose (or more generally energy) content in the upper intestinal lumen fluid and modulate the glucose-sensitive insulin secretion by the β cells in the endocrine pancreas (Ebert & Creutzfeldt, 1987; Roberge & Brubaker, 1993; Thorens & Waeber, 1993).

The existence of specific nutritional intestinal signals for Na had been controversial (Carey, 1978; Obika *et al.* 1981). Recently, the presence of Na intestinal signals was found in normotensive rats and it was suggested that their absence in hypertensive rats may be the cause of Na dependent hypertension (Mu *et al.* 1995). Thus, it is possible that the Na intestinal signals are amenable to permissive mutations and are species dependent.

The present study was initiated by the hypothesis that intestinal signals may exist for phosphate, owing to its central place in biology as a component of the genome, its function in energy transformation processes and its role as a major constituent of the skeleton of vertebrates. Since the complex process of growth is affected also by the omission and restoration of a single essential component in the diet (Dorup *et al.* 1991; Flyvbjerg *et al.* 1992; Orentreich *et al.* 1993), the present study was undertaken in order to characterize dietary phosphate-dependent growth in juvenile rats. The aim was to determine whether cessation and restoration of growth and/or appetite following alterations in phosphate content in the diet are contingent on changes in the phosphate level in the interstitial fluid. The results of the present study suggest the existence of phosphate signals emanating from the digestive system that affect the growth of juvenile rats.

Abbreviations: LPD, low-phosphate diet; NPD, normal-phosphate diet; PTH, parathyroid hormone.

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Materials and methods

Animals

Sprague–Dawley male rats weighing 140–160 g (age about 6 weeks) were used. The weight at day 0 was considered as 100 %. Growth rates were calculated as %/d, i.e. 1 %/d is equivalent to 1·4–1·6 g/d. Unless otherwise specified, rats were housed individually in cages and body weight, food consumption and water intake were determined.

Diets

The chow consisted of low-phosphate diet (LPD; P content 0·02–0·04 %) obtained from ICN Pharmaceutical Inc. (Costa Mesa, CA, USA). This diet contained 20 % (w/w) bovine blood fibrin as a protein source, 0·41 % (w/w) Ca, (with no phytic acid) and vitamin D at 325 μg/kg. Other essential minerals were prepared from phosphate-free salt mixture and are specified in the ICN Pharmaceutical Inc. catalogue. This chow contained about 10 % (w/w) water.

The drinking water was prepared from demineralized water and contained either NaCl and KCl 22·5 mmol/l each or phosphate at 25 mmol/l, pH 7·4, with Na^+ and K^+ as cations at 22·5 mmol/l each. These solutions are referred to as the Cl^-- or phosphate-containing drinking water respectively. Additional groups of rats were raised on normal animal-house diet containing (w/w) 0·57 % P (NPD). This NPD contained (w/w) 20 % protein, 0·65 % Ca and 75 μg cholecalciferol/kg. The water content in NPD was about 10 % (w/w).

The use of 25 mmol phosphate/l in the drinking water was chosen because our preliminary experiments indicated that this concentration was just above the minimal phosphate concentrations needed to restore growth in LPD-fed rats by approximately 100 %. As indicated on p. 219, P intake under these conditions was about 50 % of the nominal P intake in the NPD-fed rats.

Experimental procedures

The groups of rats used in the present study were as follows.

Group A1. This group consisted of twenty-four rats that were raised on LPD and started with Cl^--containing drinking water. At day 5 the Cl^- in the drinking water was changed to phosphate for up to 12 d. Six rats were withdrawn at days 5, 6, 7 and 12 of the experiment for the analysis of blood and tissue as described later.

Group A2. This group consisted of twelve NDP-fed rats that were provided with Cl^--containing drinking water. Six of these rats were killed after 5 d and the remaining six rats after 12 d.

Group A3. This group consisted of six control NPD-fed rats that had their drinking water changed from Cl^- to phosphate on day 5.

Group B. This group consisted of thirty rats that were raised on LPD for 3 d. At the start of day 4 the drinking water was changed from the Cl^-- to the phosphate-containing solution. Three rats were killed just before the change to phosphate, and thereafter groups of three rats each were examined at about 1, 2, 3, 5, 7, 10, 13, 17 and 21 h after phosphate supplementation. The rats in this experiment were housed in groups of three and, therefore, the data on their food and water intake represents the mean values for three rats.

Group C1. This group consisted of six rats raised on LPD and phosphate-containing drinking water for 12 d.

Group C2. This group consisted of six rats raised on LPD and phosphate-containing drinking water for 6 d. Thereafter the phosphate in the drinking water was replaced by Cl^- up to day 12.

Group D1. This group consisted of six rats raised on LPD and Cl^--containing drinking water for 12 d.

Group D2. This group consisted of six rats raised on LPD for 12 d. On day 6 the Cl^- in the drinking water was replaced by phosphate. The rats in this group were pair-fed with group D1 from days 6 to 12.

The experiments involving group A rats were conducted at the same time, i.e. using the same consignment of rats from the provider and identical batches of LPD diets. A similar procedure was followed for experiments involving group B, group C and group D rats.

All experiments were conducted in a room with a controlled temperature of 21–23°C and 12 h cycles of light (07.00–19.00 hours) and dark (19.00–07.00 hours). The day of each experiment started at about 13.00 hours, i.e. at this time the change of the drinking water was carried out and body weight and food and water intakes were measured. Blood and tissue samples from rats withdrawn from the experiment were taken at 10.00–13.00 hours. An exception to these procedures was that for group B, in which determinations were made at shorter intervals after zero time, which was also 13.00 hours.

Chemical analysis

Rats were killed by administration of an overdose of barbiturate. Blood samples were taken during the anaesthetic stage for the determination of phosphate, Ca and parathyroid hormone (PTH) levels in the serum. Total P content was determined in liver and brain tissues. Sera were prepared from the ice-cooled blood samples and aliquots of the samples were stored at −70°C before the determination of PTH. Serum Ca and phosphate concentrations were determined using the automated analytical system at the Hadassah University Hospital (Vitros 950 analyzer; Ortho-Clinical Diagnostics, Rochester, NY, USA), in which the inorganic phosphate is determined by measuring the coloured product of ammonium phosphomolybdate complex with *p*-methylaminophenol sulfate and Ca is determined by the spectrophotometric determination of its complex with Arsezano III (Sigma-Aldrich Fine Chemicals, St Louis, MI, USA) dye. Total P content in liver and brain samples was determined by the method of Ames (1966). PTH levels in the sera were determined by the use of the specific radioimmunoassay kit for rat PTH obtained from Nichols Inst. Diagnostics (San Juan, CA, USA).

Data analysis

All results are expressed as means with their standard

errors. The significance of differences was assessed by two-tailed *t* test. Bonferroni correction was performed whenever multiple comparisons were made. $P < 0{\cdot}05$ was considered significant.

Results

Rats raised on LPD were supplied with Cl^--containing drinking water for 5 d and then switched to phosphate-containing drinking water for another 7 d (group A1). A few rats were analysed during the experiment just before and at various time intervals after the change from Cl^-- to phosphate-containing drinking water. Control experiments were carried out with rats fed NPD (groups A2 and A3). The results of these experiments are shown in Figs. 1 and 2.

The effect of the shift from chloride- to phosphate-containing drinking water on growth in rats fed the low-phosphate diet

Replacement of Cl^- by phosphate in the drinking water led to a change in the rate of growth from 0·72 (SE 0·10) to 5·68 (SE 0·26) %/d at days 2–5 and 5–12 respectively ($P < 0{\cdot}01$) in the rats raised on LPD (Fig. 1(a)). In the first day after phosphate supplementation the rate of increase in body weight was at a peak of 7·61 (SE 0·53) %/d (which was significantly different from the average rate of growth during days 5–12 $P < 0{\cdot}01$). In the NPD-fed rats the rate of growth throughout this period remained constant at 4·32 (SE 0·34) %/d independent of the content of the drinking water. Hence, the rate of growth of the LPD-fed rats supplemented with phosphate in the drinking water was higher than that of rats raised on ordinary chow ($P < 0{\cdot}01$). This was so even though the P intake in the NPD-fed group was approximately 2 mmol/100 g per d (food intake of about 10 g/100 g per d containing 18 mmol P/100 g; Fig. 1(b)) whereas the phosphate intake through the drinking water in

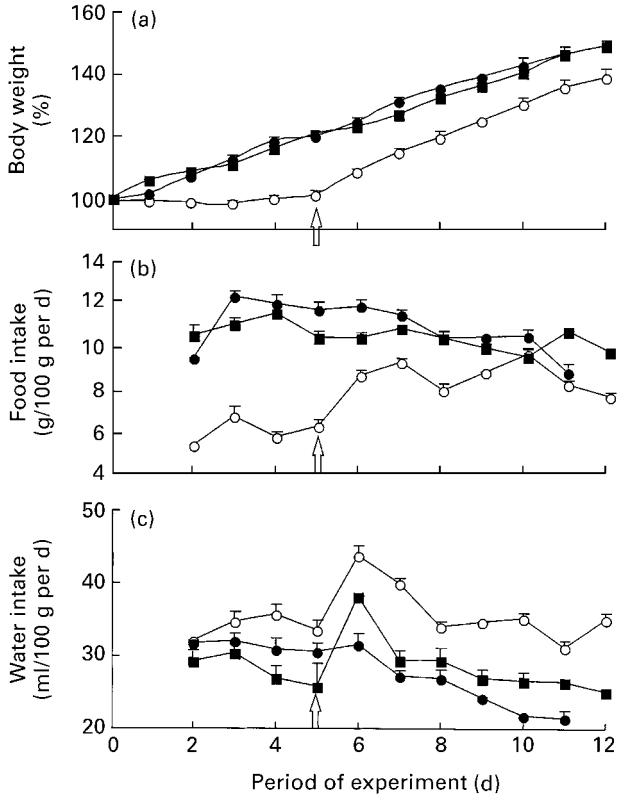

Fig. 1. Effects of phosphate supplement on growth and on food and water intakes. Body weight (a), food intake (b) and water intake (c) of rats raised on a low-phosphate diet (LPD), group A1 (○), and on a normal-phosphate diet (NPD), groups A2 (●) and A3 (■). At day 5 (⇑) the chloride in the drinking water was changed to phosphate for groups A1 and A3. Group A2 remained on chloride-containing drinking water throughout the experiment. For details of diets, drinking water and procedures, see p. 218. Values are means with their standard errors represented by vertical bars. Group A1, *n* 24 for days 0–5, *n* 18 for day 6, *n* 12 for day 7 and *n* 6 for days 8–12. Group A2, *n* 12 for days 0–5 and *n* 6 for days 6–11. Group A3, *n* 6.

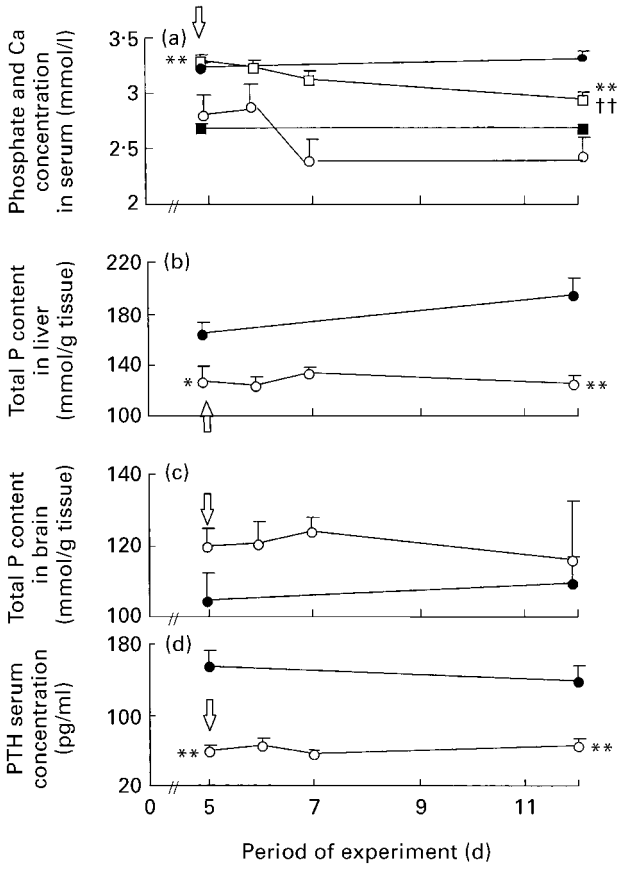

Fig. 2. Effects of phosphate deprivation and supplementation on the phosphate, Ca and parathyroid hormone (PTH) levels in the serum and on the phosphorus content in the liver and the brain. Phosphate (○, ●) and calcium (□, ■ squares) serum concentration (a), total phosphorus content in the liver (b) and the brain (c), and PTH serum concentration (d) in rats raised on a low-phosphate diet group A1 (○, □) and normal-phosphate diet (NPD) group A2 (●, ■). At day 5 (⇓) the chloride in the drinking water was changed to phosphate for group A1. For details of diets, drinking water and procedures, see p. 218. Points represent the means of analyses performed on six rats with their standard errors represented by vertical bars. Mean values were significantly different from those for NPD-fed rats; $*P < 0{\cdot}05$, $**P < 0{\cdot}01$. Mean value was significantly different from that for day 5: $††P < 0{\cdot}01$.

the LPD-fed rats was less than 1·1 mmol/100 g per d (water intake of 32–44 ml/100 g per d of a solution containing 25 mmol phosphate/l; Fig. 1(c)).

The effect of the shift from chloride- to phosphate-containing drinking water on the food consumption in rats fed the low-phosphate diet

Food consumption in the rats raised on LPD increased from 6·61 (SE 0·27) to 9·02 (SE 0·25) g/100 g per d ($P < 0·001$) on the switch from the Cl^-- to the phosphate-containing drinking water (Fig. 1(b)). This change in food intake was close to the maximum value at the first day after the change from the Cl^-- to the phosphate-containing drinking water.

The effect of the shift from chloride- to phosphate-containing drinking water on water intake in rats fed the low-phosphate diet

The switch from Cl^-- to phosphate-containing drinking water was accompanied by a transient increase in water consumption that peaked after the first day, e.g. from 34·7 (SE 1·29) to 43·8 (SE 1·34) ml/100 g per d ($P < 0·001$; Fig. 1(c)). This transient increase in water intake occurred also in rats maintained on NPD (group A3), i.e. from 27·7 (SE 1·61) to 38·2 (SE 3·22) ml/100 g per d ($P < 0·01$; Fig. 1(c)).

The effect of the shift from chloride- to phosphate-containing drinking water on phosphate, calcium and parathyroid hormone levels in the serum and on phosphate content in the brain and the liver of rats fed the low-phosphate diet

There were no significant changes in phosphate concentration in the serum following the shift from Cl^-- to phosphate-containing drinking water in the LPD-fed rats. The phosphate concentration varied from 2·83 (SE 0·17; *n* 9) to 2·85 (SE 0·20; *n* 9), to 2·41 (SE 0·20; *n* 6) and to 2·41 (SE 0·18; *n* 6) mmol/l after 1, 2 and 7 d, respectively (Fig. 2(a)). This finding implies that the restoration of growth (Fig. 1(a)) was not mediated by an increase in the concentration of phosphate in the extracellular fluid.

The Ca level in the serum of the LPD-fed rats was, as expected, higher than that of the NPD-fed rats, e.g. 3·28 (SE 0·049) *v.* 2·69 (SE 0·033) mmol/l $P < 0·01$; Fig. 2(a)). This difference was associated with a lower PTH level in the LPD-fed rats compared with the NPD-fed rats, i.e. 59 (SE 5) *v.* 158 (SE 14) pg/ml ($P < 0·01$; Fig. 2(d)). The supply of phosphate in the drinking water was accompanied by a slow decrease in serum Ca concentration that did not reach the level of the NPD-fed rats even after 7 d (Fig. 2(a)). The PTH level remained low and unchanged throughout the 7 d of growth following the switch to phosphate-containing drinking water (Fig. 2(d)) in spite of the decrease in serum Ca from 3·28 (SE 0·049) to 2·93 (SE 0·062) mmol/l ($P < 0·01$; Fig. 2(a)). These results suggest that the dietary phosphate-dependent growth was not contingent upon changes in Ca or PTH serum concentrations.

The total P content in the soft tissues such as liver and brain did not change following the addition of phosphate to the drinking water (Fig. 2(b) and (c)). In the liver the P content was significantly lower in the LPD-fed rats compared with the NPD-fed rats ($P < 0·05$) and it remained so even after 7 d of a phosphate supplement ($P < 0·01$; Fig. 2(b)). In the brain, on the other hand, the P content was the same or even a little higher in the LPD-fed rats compared with the NPD-fed rats (Fig. 2(c)).

The effect of the shift from chloride- to phosphate-containing drinking water on growth and on phosphate and calcium serum concentrations in rats fed the low-phosphate diet: fast time analysis

These experiments were conducted in order to exclude the possibility that the restoration of growth after the addition of phosphate to the drinking water was preceded by a transitory increase in the phosphate level in the serum. The results of this experiment are shown in Fig. 3. It can be seen that there was no significant increase in phosphate or decrease in Ca serum concentrations at any time during the 21 h following the change to phosphate-containing drink-

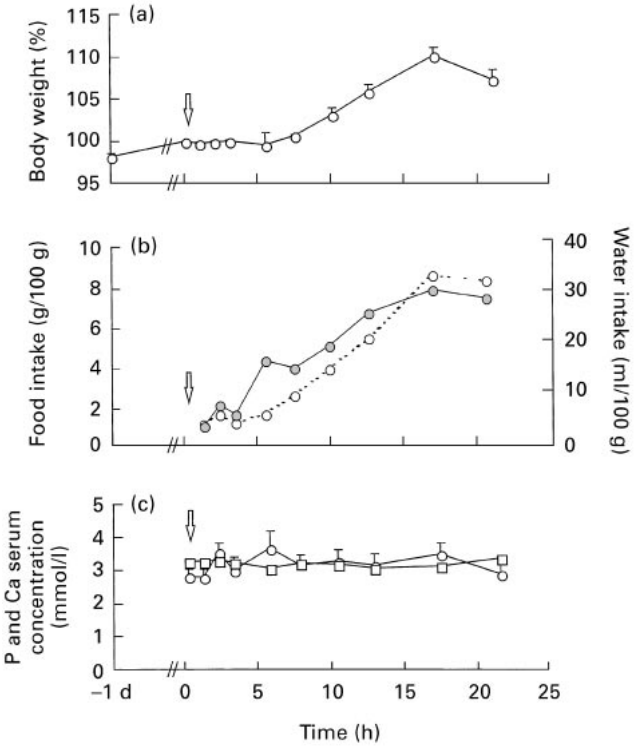

Fig. 3. Short-term effects of phosphate supplementation on phosphate and calcium serum concentrations, on body weight and food and water intakes. The chloride-containing drinking water of thirty rats raised on a low-phosphate diet for 3 d (group B) was changed at time zero (⇓) to a phosphate-containing solution. Body weight (a), food (○---○) and water (◎–◎) intakes (b) and serum phosphate (○) and calcium (□) concentrations (c) of the rats are shown at various intervals after the shift to phosphate-containing drinking water. The body weight in the preceding day (−1 d) is shown for the thirty rats. Points are means with their standard errors represented by vertical bars for three rats except for food and water intakes where only the mean intakes for groups of three rats were measured. Zero time was at 13.00 hours. For details of diets, drinking water and procedures, see p. 218.

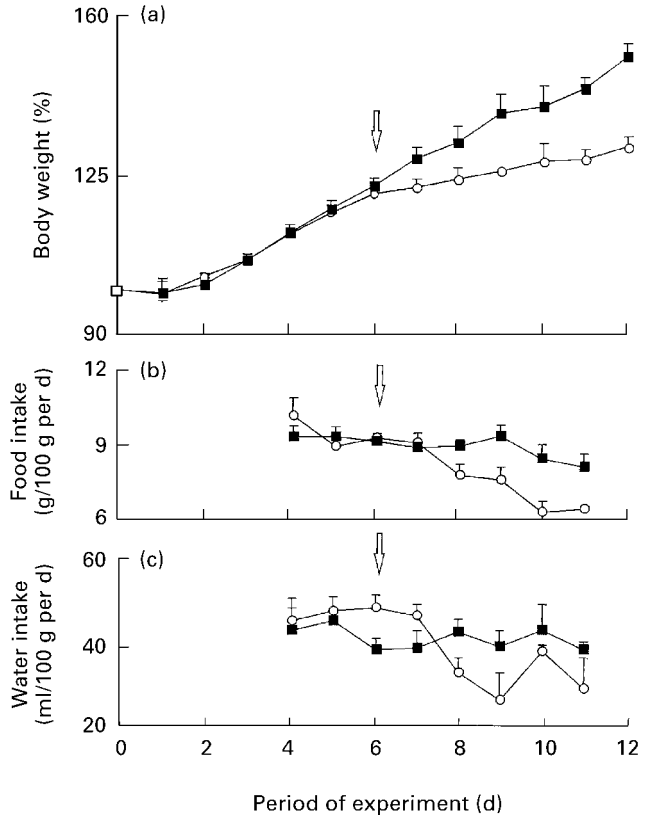

Fig. 4. Effects of phosphate deprivation on growth (body weight; a) and food (b) and water (c) intakes of rats fed a low-phosphate diet. The rats were provided with phosphate-containing drinking water for 12 d, group C1 (■), or for only 6 d following which the phosphate in the drinking water was replaced by chloride (⇓), group C2 (○). Points represent the means with their standard errors represented by vertical bars for six rats. For details of diets, drinking water and procedures, see p. 218.

ing water. On the other hand, the increase in body weight was already apparent after about 9 h following that change. Thus, there is no evidence for a supposition that transitory changes in serum phosphate or Ca concentrations might mediate the growth response to the restoration of phosphate supply.

The effect of switching from phosphate- to chloride-containing drinking water on rats maintained on the low-phosphate diet

In these experiments, two groups (C1 and C2) of rats were raised on LPD. Both groups started with phosphate-containing drinking water. In group C2 the drinking water was changed after 6 d to Cl^--containing drinking water for the remaining 6 d of the experiment. The results of this experiment are shown in Fig. 4.

The effect of the shift from phosphate- to chloride-containing drinking water on growth and on food and water consumption in rats fed the low-phosphate diet

On replacing phosphate with Cl^- in the drinking water of the LPD-fed rats, there was a clear retardation of growth from 4·42 (SE 0·59) to 1·71 (SE 0·21) %/d ($P < 0·01$; Fig. 4(a)). The change from phosphate- to Cl^--containing drinking water was accompanied by a decrease in food consumption from 8·51 (SE 0·21) to 6·75 (SE 0·12) g/100 g per d, ($P < 0·01$; Fig. 4(b)). The time course of the changes in the food intake and the rate of growth were different. The decrease in the growth rate was evident within 1 d after the change to Cl^--containing drinking water, whereas the decrease in food intake occurred only after 2 d and reached its minimum only after 4 d (Fig. 4(a) and (b)).

The change from phosphate- to Cl^--containing drinking water was accompanied by a decrease in water intake from about 45 to about 30 ml/100 g per d (Fig. 4(c)). The absolute values of the water intakes were well above the changes in body weight. Thus, it is unlikely that the latter arose simply from the changes in water consumption.

The effect of the shift from phosphate- to chloride-containing drinking water in rats fed the low-phosphate diet on phosphate and calcium serum concentrations

After 6 d following the change from phosphate- to Cl^--containing drinking water there was only a non-significant decrease ($P > 0·05$) in the phosphate serum concentration, e.g. from 2·28 (SE 0·12) in group C1 to 1·9 (SE 0·16) mmol/l in group C2. This effect is clearly different from the very pronounced effect that the removal of phosphate had on the growth rate (Fig. 4(a)). Thus, it is in accord with the conclusion drawn previously that the dietary phosphate-dependent growth was not mediated by changes in the phosphate level in the extracellular fluid. The Ca serum concentration increased slightly on the change from phosphate- to Cl^--containing drinking water, e.g. from 3·07 (SE 0·071) (group C1) to 3·25 (SE 0·015) mmol/l (group C2; $P < 0·05$).

The effect of pair-feeding on the growth response and phosphate and calcium plasma concentrations

In these experiments two groups (D1 and D2) of rats were raised on LPD for 12 d. Both groups started with Cl^--containing drinking water. In group D2 the drinking water was changed on day 6 to phosphate-containing drinking water for the remaining 6 d of the experiment. After the change to phosphate-containing drinking water the rats in group D2 were pair-fed with group D1. The results of this experiment are summarized in Table 1. It can be seen that, even though the phosphate-supplemented group was partially starved, the rate of growth was significantly higher than that in the phosphate-deprived group ($P < 0·01$). However, unlike the group with unrestricted food intake (Fig. 2(a)), phosphate supplementation in the pair-fed group led to a clear increase in plasma phosphate concentration (Table 1).

Discussion

The fact that the omission of a single essential ingredient from the diet can lead to retardation of growth is both plausible and well known (Dorup *et al.* 1991; Flyvbjerg

Table 1. Effect of pair-feeding on growth after phosphate supplementation in the drinking water in rats fed a low-phosphate diet (LPD) and on serum phosphate and calcium concentrations determined at the end of the experiment†

(Mean values with their standard errors for six rats)

Treatment group	D1		D2		Statistical significance of difference between treatment groups
	Mean	SE	Mean	SE	
Serum phosphate, day 12 (mmol/l)	2·19	0·14	3·57	0·16	**
Serum Ca day 12 (mmol/l)	3·18	0·032	2·65	0·071	**
Growth rate for days 7–12 (%/d)	1·30	0·15	3·21	0·23	**
Food intake for days 7–12 (g/100 g per d)	6·96	0·21	6·96‡		

D1, LPD-fed rats receiving chloride-containing drinking water for 12 d; D2, LPD-fed rats receiving chloride-containing drinking water on days 0–6 and phosphate-containing drinking water on days 6–12 and pair-fed with group D1.

** $P < 0{\cdot}01$.

† For details of diets, drinking water and procedures, see p. 218.

‡ Pair-fed with group D1.

et al. 1992; Orentreich *et al.* 1993). It is, however, interesting to compare the growth retardation effects due to elimination of phosphate from the diet to the similar effects that were brought about by the omission of other ions such as Ma^{2+}, Zn^{2+} and K^{+}.

Mg deprivation starts to affect growth rate by about 50 % only after its serum concentration has declined by 70 %, e.g. from 0·75 to about 0·25 mmol/l (Dorup *et al.* 1991). Mg repletion leads to restoration of the growth rate, but only after a concomitant overshoot increase in the serum Mg concentration to more than 1·0 mmol/l (Dorup *et al.* 1991). Similarly, elimination of Zn from the diet leads to a severe decline in the growth rate, but this is accompanied by a pronounced decrease in the serum Zn concentration from 20 to 7 μM (Dorup *et al.* 1991). The resumption of growth subsequent to the addition of Zn occurs following a return of the serum Zn level to the initial value (Dorup *et al.* 1991). In rats receiving a K-depleted diet growth rate is decreased by >65 %. Food consumption decreased by about 25 % with a time constant of about 3 d and was accompanied by a decrease of about 40 % in the serum K concentration, with a time constant of about 1 d (Flyvbjerg *et al.* 1992). In contrast, in our study a decrease of about 64 % in growth rate in phosphate-depleted rats (Fig. 4(a)) was accompanied by an non-significant 17 % decrease in serum phosphate concentration (see p. 221). The restoration of growth rate after inclusion of phosphate in the drinking water was associated with non-significant changes in serum phosphate concentration (Figs. 1(a) and 2(a)). Furthermore, our results show that there was no significant increase in serum phosphate concentration at any time point during the course of the first day after the shift from Cl^{-}- to phosphate-containing drinking water (Fig. 3).

The most plausible explanation for this unique phosphate-dependent growth is that intestinal receptors, which monitor the phosphate content in the digestive tract, are responsible for sending humoral or neuronal signals that affect growth. Further studies are required to determine whether the phosphate intestinal signals that influence growth are humoral or nervous or both. It will also be of interest to investigate whether these or analogous signals play a role in determining the renal handling of phosphate and/or in the regulation of 1α, 25-dihydrocycholecalciferol production (Trohler *et al.* 1976; Caverzasio & Bonjour, 1985; Levi *et al.* 1994).

It is interesting to note, also, that the brain P content of the LPD-fed rats seemed to be a little higher than that of the NPD-fed rats (Fig. 2(c)). This finding suggests that Na–phosphate transporters in the brain (Furman *et al.* 1997) were up regulated or activated by the LPD, as in the kidney (Murer *et al.* 1991; Levi *et al.* 1994).

As with other factors, the decrease in growth is associated with a decrease in food intake in the rats fed *ad libitum* (Flyvbjerg *et al.* 1992). In all these situations the question arises as to whether the primary effect is on growth, and the food consumption is only secondarily affected, or vice versa, i.e. the main effect is on appetite and growth is affected subsequently, owing to nutritional deprivation. It is also possible that the signals influence in parallel both food intake and growth. Analysis of the time course of the previously mentioned variables, following phosphate withdrawal or addition to the drinking water, revealed asymmetric responses. Withdrawal of phosphate led to the maximum decrement in growth in the first day, whereas the decrease in food intake reached its minimum only after 4 d (Fig. 4(a) and (b)). On the other hand, phosphate addition led to an immediate increment in body weight that was associated with an equally rapid effect on food intake (Fig. 1(a) and (b)). Note that the same asymmetry occurred also with respect to water intake. In the phosphate-to-Cl^{-} change there was a gradual decrease in water intake (Fig. 1(c)), whereas following the change from Cl^{-} to phosphate there was a transitory peak in water intake in the first day (Fig. 1(c)). The fact that in the pair-fed group the growth during a period of 6 d was still apparent even with food restriction (Table 1) suggests that in the change from Cl^{-} to phosphate there was still a growth effect that was independent of food intake. It is indeed possible that part of the robust growth effect seen in the first day after phosphate addition (Fig. 1(a) and p. 219) was due also to the transitory increment in water intake (Fig. 1(c)) and the accompanying increase in food intake.

The phosphate supplement under the condition of food restriction led to a rise in phosphate concentration in the plasma (Table 1), unlike the situation in the group fed *ad libitum* (Fig. 2(a)). This fact may represent one of, or both, the following factors. First, with food restriction the growth rate was lower than that in the rats fed *ad libitum* and, therefore, the phosphate intake exceeded the quantity needed for body growth. Second, the condition of pair-feeding creates partial starvation, and the latter is

known to result in phosphate movement from the intracellular compartments into the interstitial fluid (Muhlbauer *et al.* 1987).

The conclusions in the present study were drawn from the comparisons of rats raised on the same low-phosphate diet while receiving phosphate or Cl^- in the drinking water. The use of NPD-fed rats in the present study was only for the purpose of orientation. No strict conclusion can be drawn from comparisons between LPD- and NPD-fed rats, since the content of these diets in constituents other than phosphate were not identical. Nevertheless, two points stand out from the comparison between LPD- and NPD-fed rats: (1) the surge in water intake after the change from Cl^-- to phosphate-containing drinking water occurred also in the NPD-fed group (Fig. 1(c)). Thus, it appears that the transient increase in water intake was based on taste cues that are largely or entirely independent of the phosphate status of the animal; (2) the higher rate of growth in the phosphate-supplemented LPD-fed rats in comparison with the NPD-fed group (Fig. 1(a) and p. 219) is probably analogous to the well-known higher growth rate seen after periods of growth arrest due to food deprivation (Keesey & Hirvonen, 1997).

A paper by Sweeny *et al.* (1998) on phosphate appetite in juvenile rats contains data that are relevant to the present study. Careful analysis of their paper shows that, after an optional supply of phosphate in the drinking water to LPD-fed rats, there was an increase in the growth rate by about 400–500 % (Fig. 1 of Sweeny *et al.* 1998), yet the phosphate concentration in the plasma after 5 d (Fig. 6 of Sweeny *et al.* 1998) was about the same as that of the phosphate-deprived rats (Fig. 5 of Sweeny *et al.* 1998). Also, following phosphate deprivation, there was an immediate halt in growth (Fig. 1 of Sweeny *et al.* 1998), although the phosphate plasma concentration declined only on the second day of the phosphate removal (Fig. 5 of Sweeny *et al.* 1998). Thus, the results of that study support our main conclusion that dietary phosphate-dependent growth is not mediated by changes in the phosphate concentration in the extracellular fluid. The most likely explanation for this phenomenon is that signals that arise from phosphate-sensitive cells in the digestive system are part of the growth mechanism of the rat.

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