

Plasma calcitonin and parathyroid hormone levels in growing pigs on different diets. II. Low calcium diet.

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Summary. Four 12-week old growing pigs were fed a control diet (0.5 p. 100 calcium) and then a low calcium diet containing 0.1 p. 100 calcium. Two hours after the morning meal and 5 hours later in the afternoon, plasma concentrations of calcium, magnesium, inorganic phosphorus, calcitonin and parathyroid hormone (PTH) were studied during the control period and during the 6 weeks of low calcium diet.

Samples were collected in four 10-day periods, one during the control diet and 3 after introducing the low calcium diet (low Ca 1, low Ca 2 and low Ca 3) ; only 7 days of each period were used for statistical analysis.

Plasma calcium and calcitonin (CT) levels were significantly decreased and urinary hydroxyproline was increased during the low Ca diet.

The appearance of tetany crisis (just before low Ca 2) was concomittant with a slight but significant decrease in plasma PTH levels.

Plasma magnesium level was more elevated during the periods 2 and 3 of low Ca than during low Ca 1 or control periods. No rise in plasma PTH level occurred during the low calcium diet ; this fact has been discussed in relation to dietary vitamin D or magnesium content.

The absence of change in plasma PTH levels in response to the slight decrease in calcemia (12 p. 100 maximum) suggested that pigs are able to adapt to unbalanced calcium-phosphorus diets. However, bone disturbances (nasal septum and mandible) were observed as well as increased levels of urinary hydroxyproline, which indicate that the use of plasma calcium and PTH levels were not sufficient for determining the severity of dietary calcium deficiency.

Our data seemed to confirm that CT in this species might play a more important role than PTH in plasma calcium regulation. Special attention has been given to the role of plasma magnesium in CT-PTH relationships.

Introduction.

In lactating cows the marked hypocalcemia occurring during parturient paresis is associated with elevated values of plasma parathyroid hormone (PTH) (Blum, Mayer and Potts, 1974 ; Blum *et al.*, 1974 ; Garel and Barlet, 1975). A rise in plasma PTH levels is also observed after intravenous infusion of EGTA or EDTA in cows (Blum and Fischer, 1975 ; Sherwood *et al.*, 1966 ; Saeki and Hayashi, 1976). However,

when dietary calcium intake is increased from 0.05 to 1.4 p. 100, neither calcemia nor plasma PTH level is changed in cows (Ramberg *et al.*, 1976.)

Histological findings in pigs have suggested that a low Ca : P ratio (0.5) induces an increase in parathyroid activity as compared with that observed using a normal Ca : P ratio (1.2) (Nuñez, Krook and Whalen, 1976) ; this agrees with a previous observation of Doige, Owen and Mills (1975). In contrast, we found no rise in plasma PTH level with a Ca-P ratio of 0.5 (Pointillart, Garel and Guéguen, 1978b) ; however, we used a dietary calcium and phosphorus intake (0.6 p. 100 Ca and 1.2 p. 100 P) different from that given by Nuñez *et al.* (1976) (0.8 p. 100 Ca and 1.6 p. 100 P) and Doige *et al.* (1975) (0.33 p. 100 Ca and 0.75 p. 100 P). According to some data, increased bone resorption was reported to result from a low dietary calcium intake (Doige *et al.*, 1975 ; Horvath and Papp, 1972 ; Stockland and Blaylock, 1973), but other authors have shown that bone composition was unchanged in such conditions (Pond, Walker and Kirtland, 1978). The latter finding suggested an adaptation of pigs to different intakes of calcium and phosphorus. The aim of the present work was to elucidate the influence of a low dietary calcium intake on plasma PTH and calcitonin (CT) concentrations in growing pigs.

Material and methods.

Animals, diets and sampling.

Four 12-week old Large-White male pigs weighing 27.8 ± 0.5 kg were used. Samples were collected in four 9 to 10-day periods, one during the control diet period and 3 after introducing the new diet. Only 7 days of each period were used for equilibrium of the variance analysis.

A chronically implanted catheter was fitted to collect blood samples twice daily from the jugular vein. The first blood sample was collected 2 hrs. after the morning meal and the second one 5 hrs. later to see if there was any meal effect or if a lag period was necessary before changes occurred. The afternoon meal was given just after the second blood puncture, i. e. 7 hrs. after the morning meal. Each sample was collected in a heparinized polyethylene tube maintained in an ice-bath. Samples intended for hormone assays were collected under peptidase inhibitors (Iniprol, Laboratoires Choay, Paris, France). After centrifugation at 4 °C, plasma samples were frozen at -30 °C until hormone assay.

During the first period the pigs were fed a control diet including 72 p. 100 maize, 26 p. 100 soya meal and 2 p. 100 mineral-vitamin mixture. This diet contained 0.52 p. 100 calcium, 0.67 p. 100 phosphorus and 0.2 p. 100 magnesium. During the next 6 weeks until slaughter, the animals received the same diet but with no calcium source in the mineral mixture, and then Ca, P and Mg contents were 0.12, 0.55 and 0.17 p. 100, respectively.

Plasma analysis.

Plasma calcium was measured by flame photometry (Eppendorf) and plasma magnesium by atomic absorption spectrophotometry (IL 151). Plasma inorganic phosphorus was estimated by colorimetry (Chen, Toribara and Warner, 1956).

Plasma CT levels were measured by radioimmunoassay in a system already described in detail (Garel, Care and Barlet, 1974 ; Garel, Savajol and Barlet, 1976). This assay involved the use of purified porcine CT (Lot K 600-072 F-2 ; 116 MRC units/mg ; Armour Pharmaceutical Co., Kankakee, Illinois, USA) for iodination through the chloramine-T method of Hunter and Greenwood (1962). The ^{125}I -labelled porcine CT was purified by gel filtration on Sephadex G-50 fine. The specific activities of 200-300 $\mu\text{Ci}/\mu\text{g}$ were achieved routinely. The same batch of purified porcine CT was also used as a standard. An antiserum (GP2₄), raised in a guinea-pig against porcine CT, was employed at a final dilution of 1 : 3 000. The tubes were incubated in an equilibrium system at 4 °C, and after 6 days were phase-separated on talcum powder. The sensitivity of the assay was 0.25 ng/ml plasma.

The plasma PTH level was also measured by radioimmunoassay using a bovine system already described (Garel and Barlet, 1976) which cross-reacted with porcine PTH. Purified bovine PTH (bPTH 1-84, a gift of J. A. Parsons and J. M. Zanelli, MRC, London, England) was labelled with ^{125}I using the chloramine-T method (Hunter and Greenwood, 1962) and purified through gel filtration on Sephadex G-50 fine. Standard curves were calibrated with pure bovine PTH (bPTH 1-84, a gift of G. D. Aurbach). An antibody, raised in a goat against partially purified bovine PTH, was employed at a final dilution of 1 : 8 000. Since it did not cross-react with the synthetic bovine 1-34 fragment, this antibody was specific against the carboxyl terminal part of the PTH molecule. After incubation for 6 days at 4 °C in an equilibrium system, the bound and the free labelled fractions were separated by adsorption on plasma-coated charcoal. The results were expressed in ng equivalents bovine PTH per ml plasma. The sensitivity of the assay was 0.3 ng/ml plasma. Inter-assay variations were excluded since all plasmas were measured in the same assay. Intra-assay variation was no more than 10 p. 100.

Urinary hydroxyproline. — Urine was collected for 2 days during each period. Hydroxyproline was determined by colorimetry (Borel, Caranjot and Jayle, 1967) after pyrrole distillation (Serafini-Cessi and Cessi, 1964).

Statistical analysis. — Several variance analyses were done with a computer in order to determine the significance of the differences observed by changing the diet, the variations between periods, the differences between morning and afternoon samples, and individual variations and interactions between the different sources of variability (pigs, days, periods, sampling hours). The method was the same as previously mentioned (Pointillart, Garel and Guéguen, 1978b), and also gave a linear correlation. All data on the figures are presented as means \pm the standard error of the means.

Results.

A small but significant decrease in calcemia (12 p. 100) was induced by the low calcium diet (figs 1 and 3). Plasma CT level was greatly decreased (35.5 p. 100) and plasma PTH level was slightly (9.2 p. 100) reduced (figs. 2, 6 and 7). An increase in magnesium (figs. 1 and 5), was observed as well as a marked elevation in urinary

hydroxyproline (32 p. 100 in the first 15 days and 73 p. 100 at the end of the experiment) (fig. 2). Phosphatemia was unchanged after introduction of the low calcium diet (fig. 1 and 4).

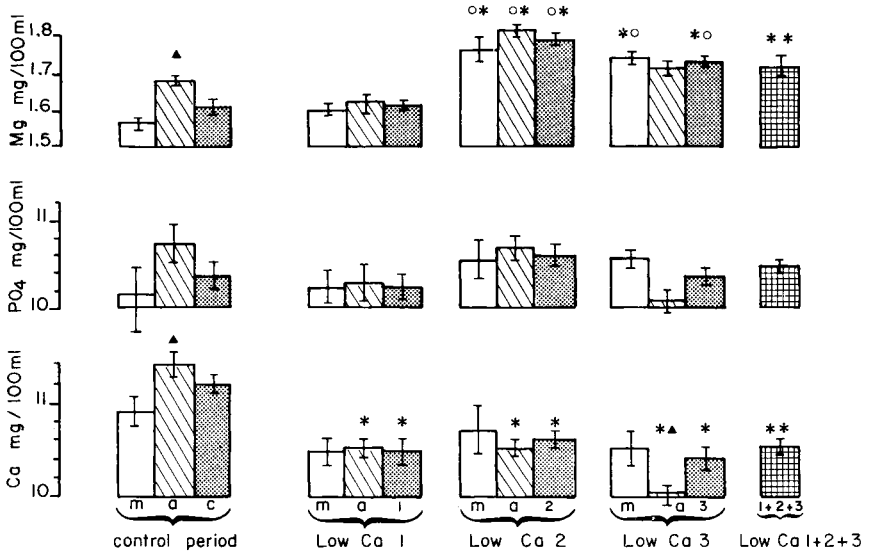


FIG. 1. — Morning (2 hrs after the meal), afternoon (5 hrs later) and daily plasma concentrations of calcium, phosphate, magnesium in growing pigs fed a control and then a low calcium diet. Period means and overall low Ca means.

Legend (fig. 1 et 2) :

- morning means ; ▨ afternoon means ; ▩ daily means ; ▧ over all low Ca means ;
- ☆ P < 0.05 (**) compared to control period (P < 0.01) ; ○ P < 0.05 compared to low Ca 1 ;
- P < 0.05 compared to low Ca 2 ; ▲ P < 0.05 compared to morning.

Differences in the parameters studied were observed during the three periods of the low calcium diet (fig. 1 and 2). During the first low-calcium period (the first 15 days), plasma CT level fell from 4.8 ng/ml (control period) to 3.3 ng/ml. Calcemia decreased (10.5 mg/100 ml vs. 11.2 mg/100 ml in the control period), but plasma PTH levels were unchanged ; plasma concentrations of magnesium and inorganic phosphorus both did not change. During the second low-calcium interval occurring after a period of tetany crisis, there was a slight decrease in plasma PTH level (2.31 vs. 2.60 ng/ml in the first period and vs. 2.65 ng/ml in the control period) and an increase in magnesemia (1.78 vs. 1.61 mg/100 ml in the first low Ca or the control period).

During the third low-calcium period (weeks 5 and 6 of the low calcium intake), the plasma CT levels remained low with values similar to those observed in the first and second low-calcium periods (3.6 vs. 4.8 ng/ml in the control period). Magnesemia was higher in the third low-calcium period than during the control period (1.73 vs. 1.61 mg/100 ml). The plasma PTH level slightly increased and recovered the

values of the control period (2.57 vs. 2.65 ng/ml in the control period). The low plasma calcium values were similar to those found in the first two intervals of low-calcium diet in spite of the long-term effect of the treatment (6 weeks).

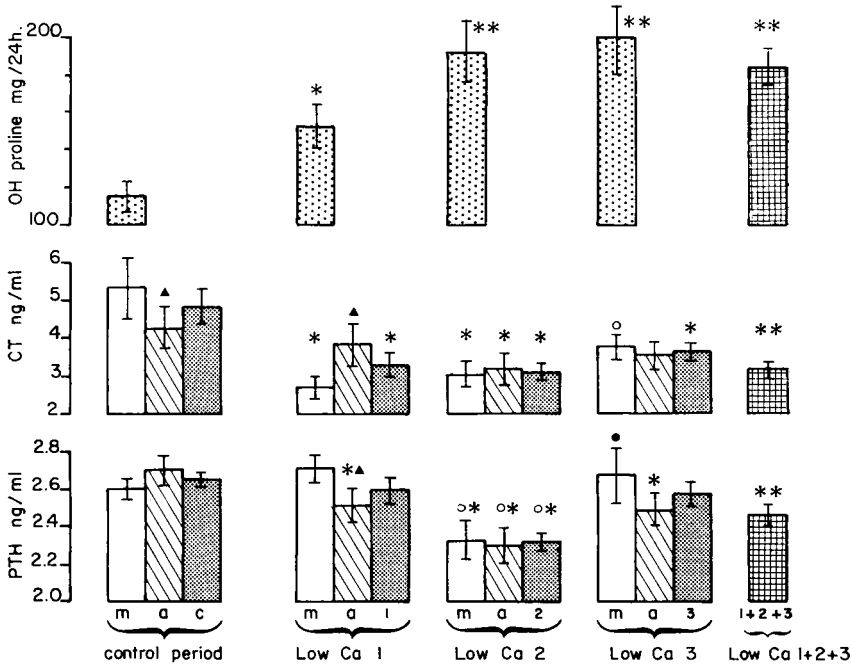


FIG. 2. — Morning, afternoon and daily plasma concentrations of calcitonin (CT), parathyroid hormone (PTH) and urinary hydroxyproline in growing pigs fed a control and then a low calcium diet. Period means and overall low Ca means.

Legend : see fig. 1. — Urinary hydroxyproline : mean values of 2 days for each period.

However, some differences were only significant for morning or for afternoon values, even if they could be significant for daily means (fig. 1 and 2). For example, morning values for plasma PTH levels increased during the third low calcium period (2.67 vs. 2.32 ng/ml in the second low calcium interval), but afternoon values for plasma PTH levels during the third low calcium period were not different from those observed in the second period (2.48 vs. 2.30 ng/ml).

The main changes introduced by the low calcium diet were a decrease in plasma calcium concentration, a fall in plasma CT level and an increase in urinary hydroxyproline. Plasma PTH levels did not increase and magnesemia was often elevated.

During the control period, plasma CT levels were higher in the morning than in the afternoon (5.3 vs. 4.2 ng/ml) (fig. 2). On the contrary, plasma calcium concentrations were higher in the afternoon than in the morning (11.4 vs. 10.5 mg/100 ml) (fig. 1). During the period of low calcium diet plasma CT level was lower in the morning than in the afternoon (2.7 vs. 3.9 ng/ml), and the opposite was observed for plasma PTH levels (2.7 vs. 2.5 ng/ml) (fig. 2).

During the control period, the plasma magnesium concentration was higher in the afternoon than in the morning (1.67 vs. 1.56 mg/100 ml, $P < 0.01$) ; this difference disappeared when the pigs were fed the low calcium diet (fig. 1). During the last 2 weeks of this diet, calcemia was lower in the afternoon than in the morning (10.03 vs. 10.50 mg/100 ml, $P < 0.05$) (fig. 2).

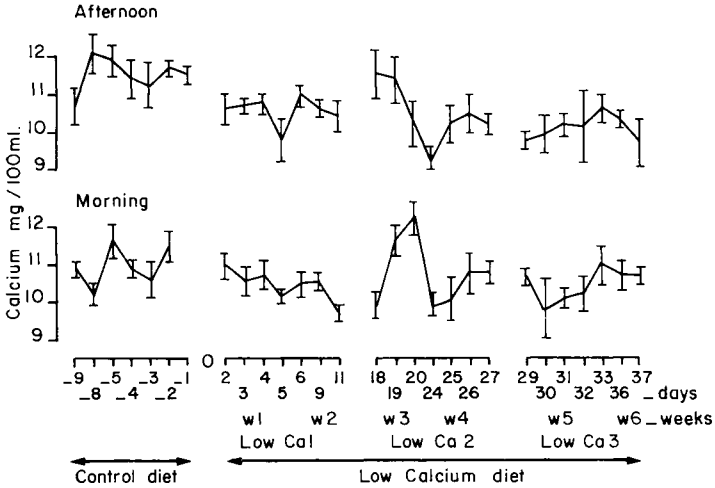


FIG. 3. — Morning and afternoon plasma concentrations of calcium in growing pigs fed a control and then a low calcium diet. Daily means.

Legend. Abcissae : number of days before (—) and after the introduction of the low calcium diet (time O).

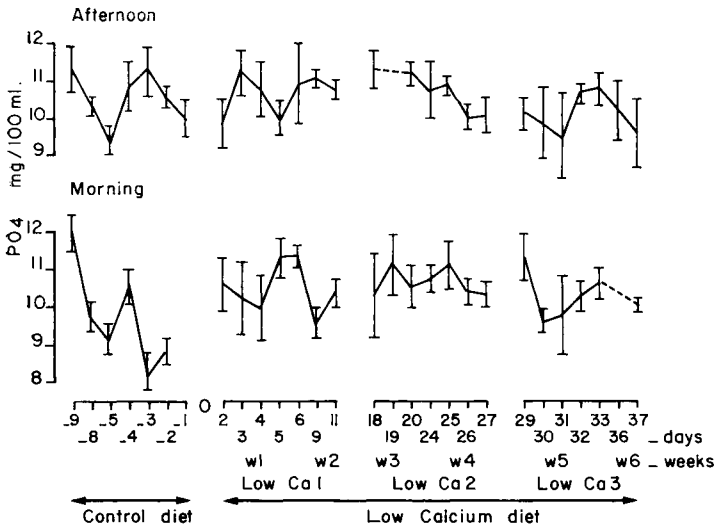


FIG. 4. — Morning and afternoon plasma concentrations of inorganic phosphorus (PO₄) in growing pigs fed a control and then a low calcium diet. Daily means.

Legend : see fig. 3.

Strong correlations did not appear whatever the period examined, but significant weak correlations were often observed, which mean that several factors were involved in the variations of one parameter. In the control period, there was a negative correlation between Mg and CT ($r = -0.51, P < 0.01$) and a positive correlation between Mg and PO_4 ($r = +0.44, P < 0.01$). During the period of low calcium diet many positive correlations appeared : Ca/ PO_4 ($r = +0.35, P < 0.01$), Ca/Mg ($r = +0.25, P < 0.05$) ; PTH/Mg ($r = +0.27, P < 0.01$). Some changes in correlation occurred between periods of observation ; a negative correlation was observed between CT and PTH during the first ($r = -0.30, P < 0.05$) and third ($r = -0.46, P < 0.01$) low calcium intervals, but this correlation was not found in the second low calcium period. A positive correlation between PTH and Mg appeared during the first and second periods ($r = +0.42$ and $+0.40, P < 0.01$, respectively) of the low calcium diet but not in the third period in which a negative correlation was found between CT and Mg ($r = -0.36, P < 0.05$) and between CT and Ca ($r = -0.36, P < 0.05$).

Pig effect was significant for all parameters studied. There were also significant pig-period interactions, except for calcemia and plasma PTH levels. The hour-period interaction was significant for plasma levels of PTH and CT.

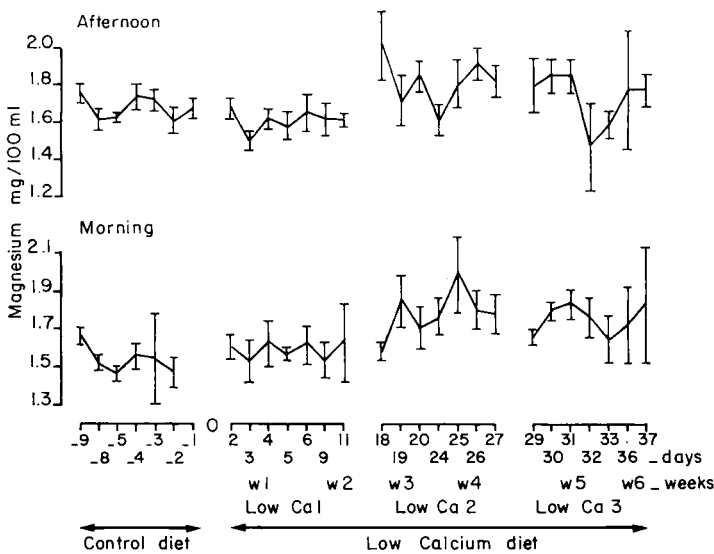


FIG. 5. — Morning and afternoon plasma concentrations of magnesium in growing pigs fed a control and then a low calcium diet. Daily means.

Legend : see fig. 3.

Great variations were observed from one day to another in the same period, particularly during the control period for plasma concentrations of calcium (fig. 3), inorganic phosphorus (fig. 4) and CT (fig. 7). Moreover, no peak was present, but a trend towards elevated plasma calcium values was observed at the end of the third

week of low calcium diet (fig. 3) after the occurrence of tetany crisis and when the lowest levels of plasma PTH were found (2.05 and 2.20 ng/ml) (fig. 6).

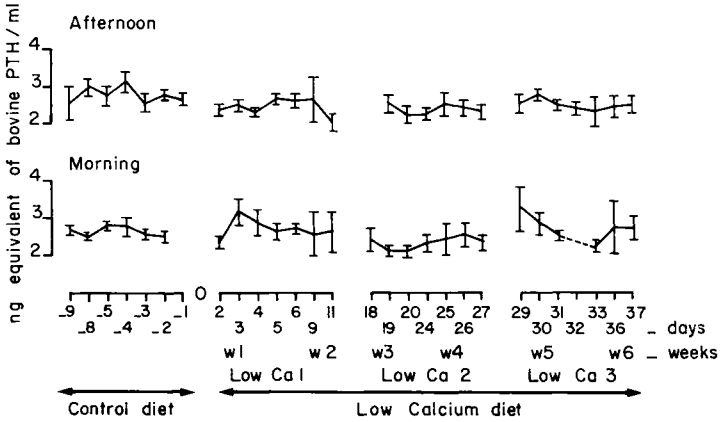


FIG. 6. — Morning and afternoon plasma concentrations of parathyroid hormone (PTH) in growing pigs fed a control and then a low calcium diet. Daily means.

Legend : see fig. 3.

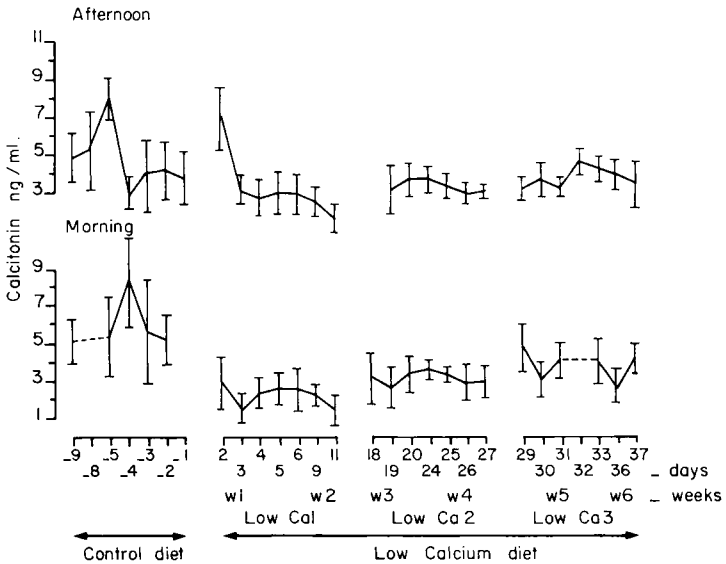


FIG. 7. — Morning and afternoon plasma concentrations of calcitonin in growing pigs fed a control and then a low calcium diet. Daily means.

Legend : see fig. 3.

Discussion.

Our results demonstrate that a low calcium diet (0.12 p. 100) induces a simultaneous decrease in plasma calcium and CT concentrations without change in plasma PTH levels. However, the marked rise in urinary hydroxyproline may suggest an increase in bone resorption.

Fox *et al.* (1977) have shown in pigs that neither PTH nor CT was necessary for intestinal adaptation to a low calcium diet since thyroparathyroidectomized pigs were able to increase their intestinal calcium absorption in response to the low calcium diet. We have observed that intestinal Ca-BP concentration was increased in pigs fed this low calcium diet (Thomasset *et al.*, 1979). The increased bone resorption suggested by the rise in urinary hydroxyproline may result from the fall in plasma CT levels. In hypocalcemic parturient cows, lowered plasma CT levels have been described by Mayer, Blum and Deftos (1975) and were associated with increased plasma PTH levels (Blum, Mayer and Potts, 1974). However, a rise in both plasma CT and PTH levels was reported by Garel and Barlet (1975) in hypocalcemic parturient cows, and Barlet (1971) has attributed parturient hypocalcemia to the stimulation of CT secretion. In the present experiments, hypocalcemia was associated with a fall in plasma CT levels, but plasma PTH levels remained unchanged. Moreover, a negative correlation was observed between Ca and CT during the third period of the low calcium diet, whereas no correlation appeared between Ca and PTH.

The appearance of tetany crisis at a time when plasma PTH levels were low suggested a disturbance in hormonal secretions. Some other factors, such as the high dietary magnesium content, may explain the low level of PTH. Elevated plasma magnesium levels are known to inhibit the secretion of PTH (Massry, Coburn, and Kleeman, 1970 ; Radde, Wittermann and Pensuwan, 1968). Thus, in the second period, when the highest plasma magnesium levels were reached, the plasma PTH levels were very low. We must also emphasize the high dietary intake of vitamin D (2 500 IU/kg) used in these experiments ; it is well-known that $1.25(\text{OH})_2\text{D}_3$ is able to stimulate bone resorption in the presence of PTH (Reynolds, 1974 ; Garabedian *et al.*, 1974). However, the dietary intakes of vitamin D_3 were the same during the control and the low calcium periods and could not explain the slight decrease in plasma PTH level occurring during the low calcium diet.

In vitamin D-deficient pigs we have observed a positive correlation between PTH levels and the length of the deficiency (Pointillart, Garel and Guéguen, 1978a). Thus, the high vitamin D intake in the present experiments may have inhibited PTH secretion. Fox *et al.* (1977) suggest that the $1.25(\text{OH})_2\text{D}_3$ alone is responsible for intestinal adaptation in pigs fed a low calcium diet. Vitamin D metabolites are known to be active in the treatment of hypocalcemia during parturient paresis (Gast *et al.*, 1977 ; Barlet, 1976, 1977 ; Sachs *et al.*, 1977). In previous work using a vitamin D-deficient diet or a high phosphorus diet, we have observed in both cases a decrease in plasma calcium concentration with no change in plasma PTH (Pointillart, Garel and Guéguen, 1978a, b), but with marked shifts in plasma CT levels. Our experiments in pigs led to the conclusion that CT secretion is more sensitive than PTH secretion to changes in plasma calcium level. Care *et al.* (1968) have already demonstrated in this species the fine responsiveness of the CT secretion rate to the blood calcium level.

The decrease in plasma calcium level is small as compared to the fall in dietary calcium intake, and this observation agrees with other results reported previously for pigs : low dietary calcium intakes (from 0.15 to 0.33 p. 100) given during 3 months did not change plasma calcium concentration (Horvath and Papp, 1972 ; Doige, Owen and Mills, 1975 ; Stockland and Blaylock, 1973).

Dietary calcium deficiency over a long period has no marked effect either on

bone calcium content or on bone ash (p. 100 dry bone). A decrease of 7.6 p. 100 in bone calcium content was observed by Horvath and Papp (1972), whereas Stockland and Blaylock (1973) found a 3 p. 100 reduction, and no change occurred according to Doige, Owen and Mills (1975) and Pond, Walker and Kirtland (1978). In our experiments, the ash content (65 p. 100 of the dry weight) and calcium content (22.7 p. 100 of the dry weight) of metacarpal bone seemed normal. Severe deformations of the nasal septum were however observed as well as brachygnathism in one animal. The last findings, combined with elevated urinary hydroxyproline, strongly suggest the presence of a marked bone resorption. In fact, neither plasma calcium levels, nor bone calcium content or plasma PTH levels are indicative of a dietary calcium deficiency in pigs. This conclusion was confirmed in the recent work of Metel (1978) in which growing pigs, fed a low calcium diet (0.3 p. 100) from 50 to 100 kg, were not hypocalcemic as compared to controls receiving 0.75 p. 100 of calcium in the diet. The ash and calcium contents of bones from pigs fed the low calcium diet were not different from those of control pigs. It is also possible that calcium or ash contents (expressed in p. 100 of dry bone) of one or some bones does not reflect the general degree of demineralization of the skeleton. Differences may be found probably between bones in the responsiveness to a low calcium diet. Even if calcium deficiency did not apparently cause any change in the degree of bone mineralization, the bone mass may be decreased. However, the present data do not permit us to draw any definitive conclusion on this question.

In pigs we must also emphasize the importance of the relationships between the plasma concentrations of magnesium and CT already observed (Pointillart, Garel and Guéguen, 1978b). In the present experiments, during the control period and the last period of the low calcium diet, an inverse relationship was established between magnesemia and plasma CT levels.

The presence of an inverse relationship between plasma CT and PTH only during the first and third periods of low calcium diet indicated some disturbance in hormonal equilibrium during the second low calcium period (after tetany crisis). It also appeared that mechanisms involved in blood calcium regulation might change throughout the period of the low calcium diet since different correlations between CT and Ca and between CT and PTH occurred during the three intervals of the low calcium diet. A similar conclusion was drawn from results obtained on pigs fed a high phosphorus diet (Pointillart, Garel and Guéguen, 1978b).

In conclusion, the low calcium diet given to young growing pigs during 1.5 months induced a small decrease in calcemia and no change in plasma PTH levels ; but plasma CT levels were greatly decreased. Our results and those from others obtained in pigs demonstrate an adaptation to unbalanced calcium-phosphorus diets. Although hormonal mechanisms are involved in the adaptation of the animals to the low calcium diet, other mechanisms (i. e. intestinal absorption) are the main factors of this response and PTH is probably marginal. The plasma magnesium level seems to play an important role in the CT-PTH hormonal equilibrium in pigs.

*Reçu en novembre 1978.
Accepté en décembre 1978.*

Acknowledgements. — We thank Dr. Duhamel for help in statistical analysis. Urinary hydroxyproline was estimated through the courtesy of Mrs. Monique Allez. Figures were constructed by Miss Catherine Briand and the animals were cared for by Messrs. Gauthier and Guichaux. Purified bovine PTH was kindly supplied by Drs. J. A. Parsons and J. M. Zanelli. We are particularly grateful to Mr. J. Choay for providing us with Iniprol. This study was partly supported by the ATP No. 2417 of the CNRS.

Résumé. Quatre porcs en croissance, âgés de 12 semaines, ont reçu un régime témoin contenant 0,5 p. 100 de calcium puis, pendant 6 semaines, un régime carencé contenant 0,1 p. 100 de calcium (BCa). Deux heures après le repas du matin et sept heures après, les teneurs du plasma en calcium, magnésium, phosphore minéral, calcitonine (CT) et parathormone (PTH) ont été mesurées durant la période témoin et durant la période BCa.

Les échantillons ont été récoltés sur 4 périodes de 10 jours environ, une durant la période témoin et trois durant la période BCa (BCa 1, BCa 2, BCa 3), les valeurs analysées portant sur 7 jours par période.

L'administration du régime carencé en calcium entraîne une baisse des teneurs plasmatiques en calcium et en calcitonine et une hausse de l'hydroxyprolinurie. L'apparition de crises de tétanie (période BCa 2) est suivie d'une baisse légère mais significative de la teneur en PTH du plasma. La magnésémie est plus élevée dans les périodes 2 et 3 du régime BCa que dans la période 1 et la période témoin.

L'absence d'élévation de la teneur en PTH du plasma, en relation avec la richesse du régime en vitamine D et/ou en magnésium et les rôles respectifs de la CT et de la PTH dans l'espèce porcine sont discutés. La baisse assez modérée de la calcémie (12 p. 100 maximum) sans réponse parathormonale est discutée en relation avec les réactions du porc aux déséquilibres phospho-calciques. Les troubles osseux constatés (cloison nasale, mâchoire) et l'augmentation de l'hydroxyprolinurie pourraient indiquer que les paramètres étudiés (Ca et PTH) ne permettent pas d'apprécier la gravité d'une carence calcique. Il semble également se confirmer que dans cette espèce la CT est plus sensible aux variations de la calcémie que la PTH.

Le rôle de la magnésémie dans l'équilibre endocrinien CT-PTH est également discuté.

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