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PLASMA LEVELS OF IMMUNOREACTIVE PARATHYROID HORMONE IN RELATION TO STARVATION HYPOCALCAEMIA IN DAIRY COWS*

By

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TOLLMAN, RICHARD L. and KAARE M. GAUTVIK: *Plasma levels of immunoreactive parathyroid hormone in relation to starvation hypocalcaemia in dairy cows.* Acta vet. scand. 1980, *21*, 457—468. — The hypocalcaemia caused by parturition and onset of lactation in high-production dairy cows was mimicked by subjecting cows to starvation periods before and after partus. The changes in plasma calcium, phosphate and magnesium were followed and compared with immunoreactive parathyroid hormone (iPTH) in 2 cows. During the starvation periods before partus, the cows developed hypocalcaemia with no or only small changes in the plasma concentration of magnesium. After the onset of hypocalcaemia, the concentration of iPTH increased on the average 3—4-fold and the raised hormone levels lasted about 24 h after start of refeeding. An increase in plasma phosphate occurred somewhat later than the rise in iPTH and lasted longer. After partus hypocalcaemia developed, together with smaller increases in iPTH concentration (about 2-fold). The post-partum starvation period again resulted in hypocalcaemia and raised iPTH concentrations.

In conclusion, starvation and parturition induced inverse changes in plasma calcium and iPTH in dairy cows. The increases in plasma iPTH were reversible and considered secondary to the hypocalcaemia. Through the effect of paratyroid hormone, plasma calcium was normalized and phosphate concentration increased. Therefore, fatal hypocalcaemia which may occur following the course of parturition and onset of lactation is not due to impaired PTH secretion.

dairy cow; starvation; parturient hypocalcaemia; parathyroid hormone; calcium; magnesium; inorganic phosphate.

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Parturition and the onset of lactation in high-producing dairy cows lead to sudden demands for large quantities of calcium. Colostral milk is especially rich in calcium (*Kronfeld & Ramberg* 1970). When milk secretion occurs simultaneously with the transient inappetence which often accompanies birth, hypocalcaemic crises such as parturient hypocalcaemia may develop.

During the last trimester of pregnancy, nearly all the calcium required by the cow and foetus is derived from dietary sources which are normally rich in calcium (*Arnaud et al.* 1971). The cows' demands for calcium are relatively small, and dietary intake easily satisfies the requirement for this cation. In this situation bone remodelling is greatly reduced and plasma parathyroid hormone (PTH) low (*Mayer et al.* 1969).

Mobilization of bone calcium in response to severe hypocalcaemia (parturition and lactation hypocalcaemia) requires an increase in the activity of the osteoclast cell population (*Rasmussen & Bordier* 1974).

In order to study the possible role played by PTH in the development of the milk fever syndrome, it is necessary to induce a hypocalcaemic condition. *Halse* (1958 unpublished) previously showed that short term starvation brought about a fall in the concentration of plasma calcium. He subsequently demonstrated that an apparent strengthening of calcium homeostasis occurred in starved cows (*Halse* 1958). In the present study we therefore subjected 2 pregnant cows to short term starvation periods before and after birth. The changes in immunoreactive PTH (iPTH) were measured and compared to changes in plasma calcium, magnesium and phosphate.

MATERIALS AND METHODS

A. *Animals*

Two "Norwegian Red" dairy cows, aged 5 and 8 years (referred to as Cow 1 and Cow 2, respectively) were used. The cows were housed indoors throughout the experiment. The animals were fed silage, hay and conventional concentrate supplement. Water was given ad libitum.

B. *Assay and analyses*

Parathyroid hormone (PTH) was assayed by a non-equilibrium radio-immunoassay as described previously (*Gautvik et*

al. 1979). The antiserum was raised in a rooster against a bovine PTH preparation (Wilson Laboratories, USA) and the resultant antiserum was directed against the N-terminal fragment (1—34) hormone (data not shown).

Radioactive PTH was prepared using ^{131}I (Amersham Radiochemicals) and iodination grade purified standard bovine PTH (Wilson Laboratories, USA). Bound and free PTH were separated with dextran coated charcoal (*Gautvik et al.*).

Standard curve analysis revealed that the lowest limit of sensitivity was 0.1 μg PTH/l. Immunoreactive parathyroid hormone (iPTH) was assayed in test plasma using 40 μl plasma aliquots in triplicate. No sample aliquot was thawed more than twice.

Each assay included standards of normo- and hyperparathyroid plasma which were interspaced between every 10th plasma sample. The intra-assay variations for a plasma with normal and high concentrations of iPTH were 19 % and 9 %, respectively. The inter-assay coefficient of variation was about 10 % for both normo- and hyper-PTH plasma.

Calcium and magnesium concentrations in plasma were determined by atomic spectrophotometry (Perkin Elmer).

Plasma inorganic phosphate was analysed by an automated colourimetric method (Technicon Instruments-File N-4c).

C. *Experimental design*

The experiment was conducted in January and February and the cows had been housed indoors for 6 months before the start of the experiment.

Starvation

The animals were subjected to a 36 h starvation period. The onset of starvation was set to be 12 h after the last feeding which occurred at 5 a.m. Food was withheld until 6 a.m., 48 h later. The 12 h period after last feed was to allow for rumen food storage depletion and represents the approximate normal time interval between 2 meals.

Cow 1 (Figs. 2 a and 2 b) was subjected to a starvation period (A) pre-partum. Before the start of the second planned starvation period calving occurred. After an 8 day rest the cow was subjected to the first post-partum starvation experiment (B). This

resulted in inappetence on refeeding and a positive milk ketone test. A second planned post-partum starvation period was abandoned. The animal was then rested and starved 17 days after the first post-partum period (C). Inappetence and a positive milk ketone test developed and further experiments were abandoned. A series of samples were taken from this animal 60 days after calving when on summer pasture.

Cow 2 (Fig. 3) was subjected to 2 pre-partum starvation periods (D, E), rested for 10 days after calving and fasted again (F). This animal ate well immediately after refeeding and was alert and active. A second post-partum experiment was therefore scheduled after a 3 day rest period, but the animal stopped eating and developed a positive milk ketone test. She then became recumbent. The experiment was immediately abandoned and treatment begun. Plasma analyses revealed severe hypomagnesaemia and hypocalcaemia which were treated twice with intravenous calcium borogluconate and magnesium sulphate. The animal died in acute cardiac failure during the second infusion.

Sampling

Blood samples were collected by jugular venepuncture into heparinized glass tubes. Plasma was prepared by centrifugation and stored at —20°C within about 1 h after collection. Prior to freezing each sample was divided into 4 aliquots to avoid repeated thawing.

Controls. Samples were collected 3 times daily for 2—3 days before the start of the first pre-partum starvation experiment, and in Cow 1, 60 days after calving.

Starvation experiments. Samples were collected thrice daily during the experiment and during the 3 day rest period between the starvation periods (D and E).

Calving. Sampling continued 3 times daily from the end of the last starvation experiment before calving, through calving and for 2 days post partum. Thereafter only 1 sample was collected each day.

Post-partum rest period. Samples were collected once daily (2 p.m.) on every second day until 3 days before the start of the first post-partum starvation experiment.

RESULTS

Starvation before calving

During starvation, the average plasma calcium concentration fell by 25 % with the lowest level (1.95 mmol/l) occurring 8 h after feed was again made available (Fig. 1). A decrease in magnesium levels occurred after starvation and reached a minimum of 0.85 mmol/l 32 h after the start of refeeding (Fig. 1). The phosphate value rose sharply towards the end, reaching a level 30 % above the initial value. It then remained elevated for the following 24 h (Fig. 1). A 3—4-fold increase occurred in plasma iPTH towards the end of the starvation period, and was maintained for 24 h after the start of refeeding (Fig. 1). Calcium,

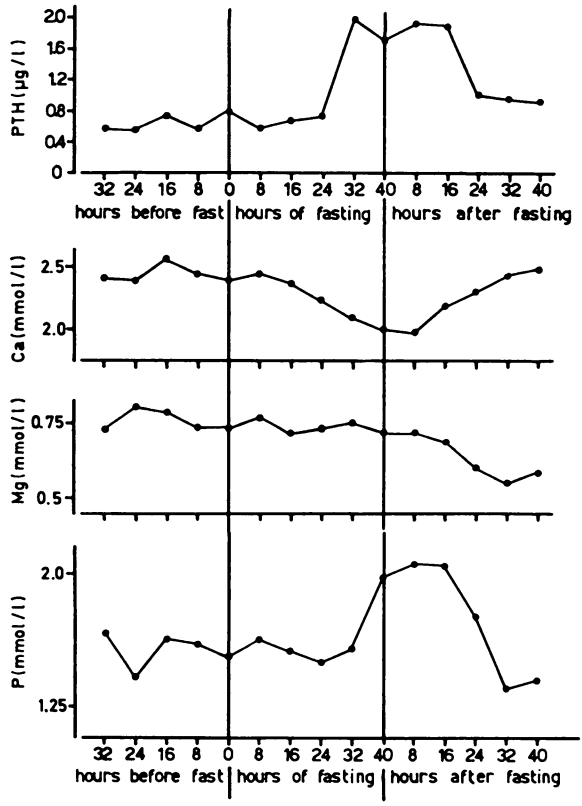

Figure 1. The combined results of 3 starvation experiments (A, D and E) performed before calving in Cows 1 and 2. The mean concentrations of parathyroid hormone (µg/l), calcium (mmol/l), magnesium (mmol/l) and plasma inorganic phosphate (mmol/l).

phosphate and iPTH returned to normal levels at about the same time after the start of refeeding, but hypomagnesaemia showed a more variable and prolonged course (Figs. 1, 2 a and 3).

Calving

In both animals calving was accompanied by transient subclinical hypocalcaemia; there was only a slight increase in iPTH associated with the hypocalcaemia of parturition (Figs. 2 a and 3).

Starvation after calving

In contrast to all other experiments performed both before and after parturition, Experiment B (Fig. 2 a) was characterized

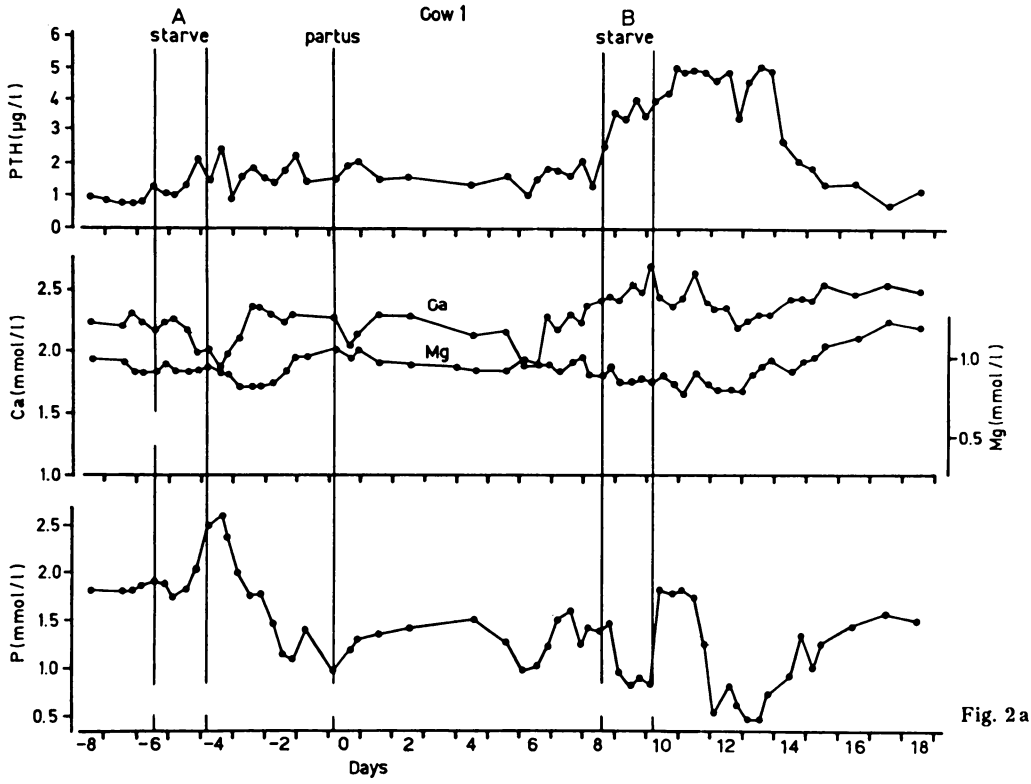

Fig. 2a

Figures 2a and 2b. The plasma levels in Cow 1 of parathyroid hormone (µg/l), calcium (mmol/l), magnesium (mmol/l) and plasma inorganic phosphate (mmol/l) during starvation experiments performed before (A) and after calving (B and C).

by an increase in the level of circulating calcium during starvation. The elevation in calcium concentrations occurred concomitantly with a 5-fold increase in the level of iPTH which paralleled the changes in calcium. Starvation was accompanied by a fall in the level of phosphate, followed by a transient rise to above the pre-starvation levels. As the iPTH concentration returned to basal, a normalization of phosphate occurred (Fig. 2 a). It is noteworthy that this experiment occurred 2 days after a transient subclinical hypocalcaemic episode in which calcium fell from 2.12 mmol/l to 1.87 mmol/l (Fig. 2 a).

In contrast to Experiment B, the starvation periods in Experiments C (Fig. 2 b) and F (Fig. 3) were accompanied by falls in calcium concentrations. In both experiments the post-starvation periods were also characterized by a severe hypomagnesaemia.

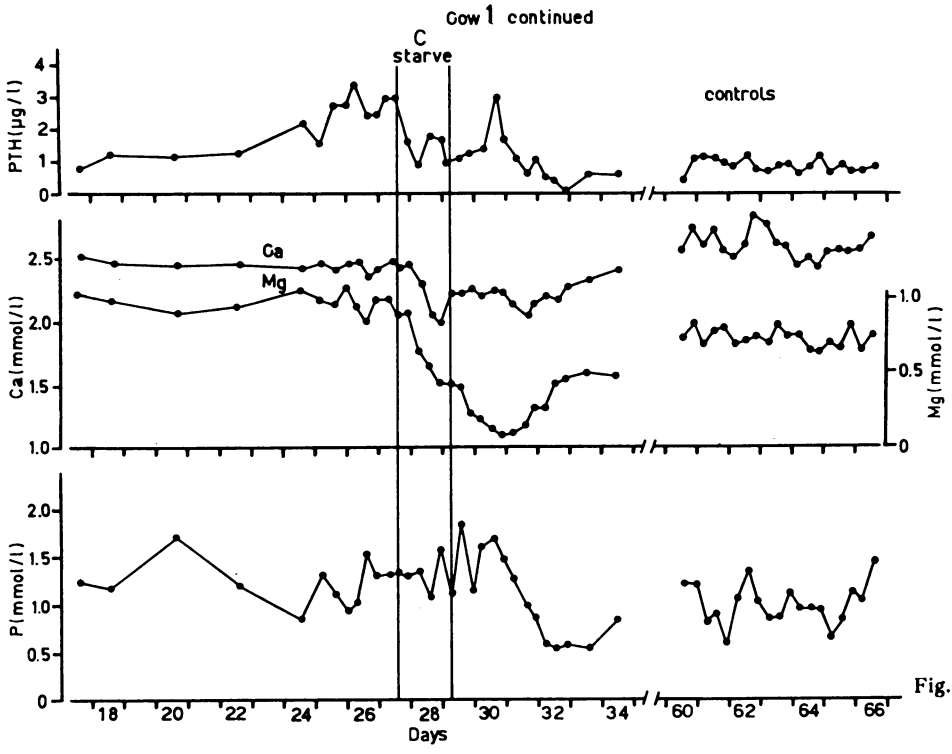

Fig. 2b

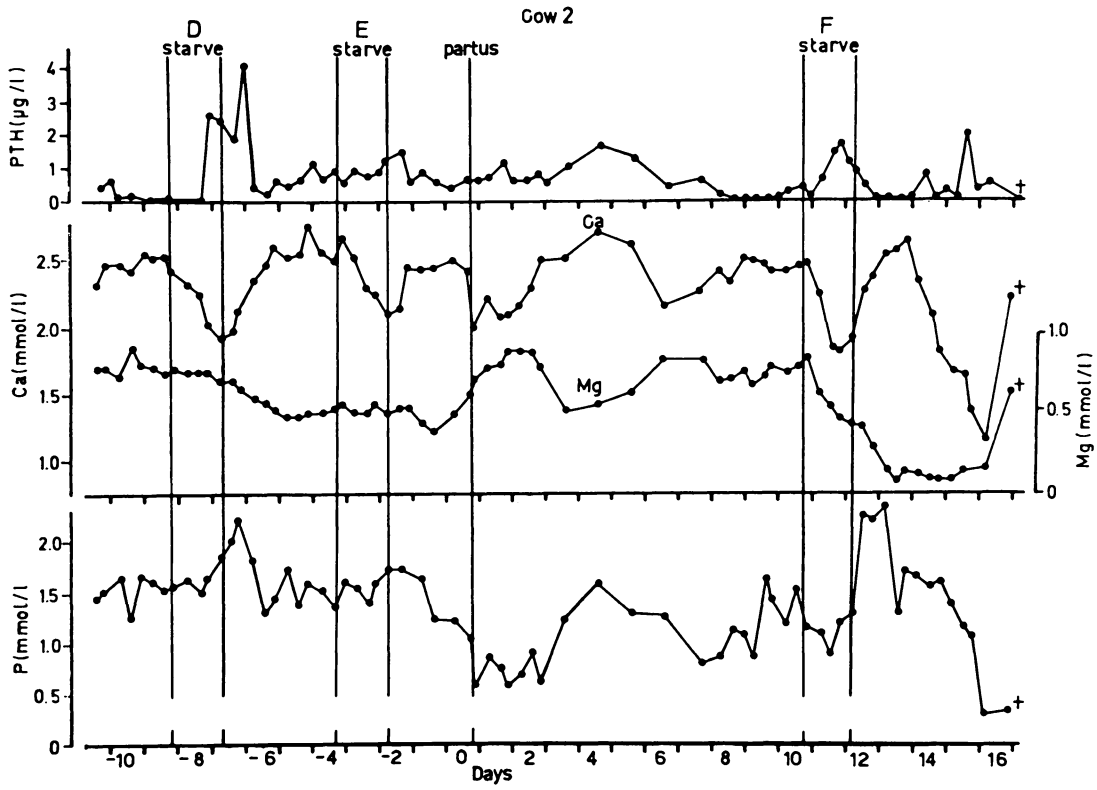

Figure 3. The plasma levels in Cow 2 of parathyroid hormone (µg/l), calcium (mmol/l), magnesium (mmol/l) and plasma inorganic phosphate (mmol/l) during starvation experiments performed before (D and E) and after calving (F). † Denotes the death of the animal.

In Cow 1 (Fig. 2 b) magnesium fell to as low as 0.31 mmol/l before recovering spontaneously. In Cow 2 (Fig. 3) magnesium fell to 0.1 mmol/l and this animal died in spite of treatment.

In Cow 2 (Fig. 3, F) the fall in calcium concentration was accompanied by an increase in iPTH, but the hormone then returned to very low values, with the exception of a transient peak occurring late in the hypocalcaemic crisis that followed starvation.

DISCUSSION

Insufficient function of the parathyroid glands has long been considered to be a contributing factor in the development of parturient paresis (*Dryerre & Grieg* 1925).

Opposed to the concept of parathyroid insufficiency are results of morphological studies which suggest functional activity of the parathyroid gland in cows with parturient paresis (*Marshak* 1957, *Capen & Young* 1967). *Mayer et al.* (1969) and *Mayer* (1970) have also found a rapid iPTH response proportional to the fall in calcium in animals with severe paresis.

In the present study short-term starvation resulted in marked changes in calcium, magnesium, parathyroid hormone and inorganic phosphate. *Sherwood et al.* (1966) reported similar results to those seen in the present experiment when studying the effect of EDTA infusion on blood calcium and iPTH concentrations. The marked hypocalcaemia and increased concentrations of iPTH that were induced during starvation, were similar to those changes seen in many dairy cows during parturient hypocalcaemic crises (*Mayer et al.*). It will be noticed, however, that starvation before parturition resulted in a larger degree of hypocalcaemia and increased iPTH concentrations than those seen during parturition in this study. The starvation experiments performed before parturition (A, D and E) might have been responsible for priming the calcium mobilizing mechanisms and thereby preparing the animal for parturition and the attendent strain of lactation. In support of this hypothesis is the observation that post-starvation calcium concentrations rose to above the levels seen at the start of starvation in Experiments A and D. Increased efficiency in the activation of iPTH secretion brought about by starvation hypocalcaemia could be, in part, responsible for this phenomenon. It will be noticed that in both Experiments A and D, the calcium overshoot occurred about 3 days after the end of starvation. This observation is in accordance with the so-called "time lag" needed for the activation of calcium defence mechanisms (*Kronfeld* 1971, *Littledyke* 1976). A time lag requirement of about 2—3 days suggests that the defence mechanisms that have to be activated require bone cell proliferation and differentiation (*Rasmussen* 1973, *Rasmussen & Bordier* 1974). The observation that feeding a low calcium diet prepartally for 10—12 days reduced the incidence of parturient hypocalcaemia (*Boda & Cole* 1954) is consistent with the results seen in the present experiments.

Starvation after parturition resulted in variable effects on plasma mineral concentrations. In Cow 1 it appears as if the priming effect of starvation before parturition remained for some

time after birth. This is indicated by the possible impact of a transient hypocalcaemic episode on serum iPTH, 6 days post partum. The starvation experiment which then followed (B) resulted in an early and sustained rise in iPTH and, contrary to all other starvation experiments, an increase in calcium throughout starvation. Phosphate fell inversely with the increase in iPTH, interrupted by a transient increase in phosphate on refeeding. The increase in phosphate seen on refeeding can be attributed to a combination of dietary phosphate and the release of tissue phosphates as a result of starvation.

Experiments C and F were performed at a time of increasing mineral demands due to lactation. In both these experiments the added load of starvation led to a dramatic fall in magnesium concentrations. Cow 2, which ultimately died, had consistently lower magnesium concentrations than Cow 1 throughout the experiment. *Halse* (1970) reported that cows with magnesium levels that were on the low end of the normal range before being put out to grass pasture, had an increased susceptibility to clinical hypomagnesaemia than cows with higher plasma magnesium concentrations.

The present experiments have demonstrated that there is no defect in the mobilization of PTH during hypocalcaemia induced by starvation or in relation to parturition. The failure to maintain plasma calcium during the strain of parturition and lactation must therefore have other explanations.

These experiments indicate that repeated stimulation of PTH secretion can increase the gland's secretory capacity and increase the functional potential of the cell population responsible for calcium mobilization from bone. However, the limited number of experiments that could be carried out and the small number of animals involved in the study, do not permit any definite conclusion as to the possible beneficial effects of a pre-partal starvation hypocalcaemia.

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SAMMENDRAG

Hypokalsemi hos melkekyr utløst ved faste.

Konsentrasjonen av parathormon (PTH), kalsium, magnesium og uorganisk fosfat ble målt i plasma hos 2 melkekyr i forbindelse med kalving og under innlagte fasteperioder.

I forbindelse med kalving økte konsentrasjonen av plasma PTH sekundært til en reduksjon i plasma kalsium konsentrasjonen. Parallelle, men mer uttalte forandringer i PTH og kalsium, ble målt under fasteperioder.

Forandringer i konsentrasjonen av magnesium og uorganisk fosfat under kalving var varierende. Under fasteperioderne var det imidlertid et fall i plasma magnesium og en bifasisk økning i plasma fosfat.

Resultatene viser at melkefeber hos kyr ikke skyldes en primær svikt i parathormon produksjon.

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