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PARTURIENT PARESIS IN THE COW

SERUM IONIZED CALCIUM CONCENTRATIONS BEFORE AND AFTER TREATMENT WITH DIFFERENT CALCIUM SOLUTIONS — CLASSIFICATION OF DIFFERENT DEGREES OF HYPO- AND HYPERCALCEMIA

By

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KVART, C., K. A. BJÖRSELL and L. LARSSON: *Parturient paresis in the cow. Serum ionized calcium concentrations before and after treatment with different calcium solutions — classification of different degrees of hypo- and hypercalcemia.* Acta vet. scand. 1982, 23, 184—196. — Serum ionized calcium concentrations (Ca_F) were determined in 87 Swedish red-and-white cows and 10 Swedish Friesian cows with clinical signs of parturient paresis. All cows were in the week prior to or after parturition. A classification of the severity of hypocalcemia in terms of serum ionized calcium was devised. Eight cows had normal serum ionized calcium concentrations (Ca_F 1.06—1.26 mmol/l); 15 had slight (Ca_F 0.80—1.05 mmol/l); 43 a moderate (Ca_F 0.50—0.79 mmol/l), and 31 a severe ($Ca_F < 0.50$ mmol/l) hypocalcemia.

All cows were given 8 or 8.3 g of calcium intravenously. Of 8 normocalcemic cows 7 (87.5 %) reached a maximum posttreatment serum ionized calcium concentration > 1.80 mmol/l (severe hypercalcemia). This was also found in 13 of 15 (86.7 %) slightly hypocalcemic cows and in 31 of 43 (72.1 %) moderately hypocalcemic cows. In the severe hypocalcemia group 14 of 31 (45.2 %) had maximum posttreatment $Ca_F > 1.80$ mmol/l).

These findings emphazise the need of a rapid pretreatment evaluation of the degree of hypocalcemia. The present study also underlined the difficulty in predicting serum ionized calcium from serum total calcium concentrations.

Swedish red and white breed; milk fever; ion selective electrode; therapeutic effect.

Parturient paresis in cattle is usually associated with hypocalcemia (*Little & Wright* 1925). The therapy of choice for parturient paresis is parenteral administration of calcium. In Sweden routine treatment usually consists of an infusion of 8 g of calcium, but this treatment is affected with different complications (*Hapke* 1972). The infusion may lead to severe hypercalcemia as one does not know the initial calcium concentration. Cardiac arrythmias occur during or after the calcium infusion and occasionally cows die during or shortly after treatment (*Hapke* 1972). This has been attributed to cardiac disturbances presumably caused by a rapid increase of the serum ionized calcium concentration (*Hapke* 1974).

In cows with parturient paresis the degree of hypocalcemia and the effect of calcium infusion in terms of serum ionized calcium concentrations have not been fully clarified. The present study was therefore performed in order to obtain information on the severity of hypocalcemia before treatment and the maximum serum ionized calcium concentration after calcium infusion.

MATERIAL

An unselected sample of 97 Swedish cows with clinical signs of parturient paresis was studied. All cows were in the week prior to or after parturation and all were of Swedish red and white breed with the exception of 10 Swedish Friesian breed (SLB). All cows came from the Fjärdhundra and Norrtälje veterinary districts in Sweden and were studied during October 1978—May 1979.

METHODS

After clinical judgement the first blood sample was drawn from one of the jugular veins before start of the intravenous calcium treatment. Four different calcium solutions (Table 1) were used in the treatment, each containing 8.0 or 8.3 g of calcium. Calcium chloride and calcium borogluconate were given with an infusion pump during 8 min. The infusion time for the commercial preparations (Cadexil® and Novocalc®) was in the range of 5—35 min. The second sample was taken immediately after cessation of the infusion (within 0.3—2 min) because frequent sampling has shown a peak serum ionized calcium concentration at that time (*Kvart & Larsson,* to be published). The post treatment blood samples were taken from the vein opposite the one used for the calcium infusion. All sampling was done with 15 ml Vacutainer® Tubes without additive (Becton Dickinson Co., Rutherford, N.J. 07070). The serum ionized calcium concentration was determined within 3 days.

Table 1. The composition of the different calcium solutions used; n indicates the number of cows treated with the respective solution.

Calcium solution	Content	
Calcium borogluconate	Calcii gluconas	90 g
(8 g of calcium)	Acid boricum	25 g
n = 15	Aq. steril ad	500 ml
Calcium chloride	Calcii chloridum sice	30 g
(8 g of calcium)	Aq. steril ad	500 ml
n = 11		
Cadexil (Pharmacia)	Calcii propionas	39 g
(8.3 g of calcium)	Magnesii chloridum	15 g
n = 33	Dextran (Pharmacia)	27.5 g
	Glucose	90 g
	Propionic acid until pH 5.4 is achieved	
	Aq. steril ad	500 ml
Novacalc (Astra Chemicals)	Calcii gluconas	16.7 g
(8 g of calcium)	Calcii lact.	11.6 g
n = 36	Calcii form.	16.5 g
	Magnesii hypophosph.	3 g
	Aq. steril ad	100 ml

Determination of serum ionized calcium (Ca_F)

Serum ionized calcium was determined with the Orion SS-20 (Orion Biomedical, Cambridge, Ma 02139) as earlier described (*Larsson & Öhman* 1978, *Öhman & Larsson* 1978, *Kvart & Larsson* 1978). Within-assay standard deviation was calculated from duplicate determinations on 73 serum samples. This parameter was 0.008 mmol/l. Between-assay variations was characterized by analyzing a control, consisting of a bovine serum pool, frozen in 5 ml aliquots at —80°C in Vacutainer tubes, at the beginning of each routine series. This control had a 0.022 mmol/l between-assay (1 s) (n = 132) during 11 months (*Larsson & Öhman* 1980). The reference range for Ca_F in the cow was 1.06—1.26 mmol/l (*Kvart et al.* 1980).

Determination of serum total calcium (Ca_T)

Serum total calcium was determined with atomic absorption spectrometry (Perkin-Elmer 460). The within-assay standard deviation was 0.019 mmol/l as calculated from duplicate determinations of 29 samples, which means a coefficient of variation (CV) of 0.8 % at 2.46 mmol/l. The between-assay precision was

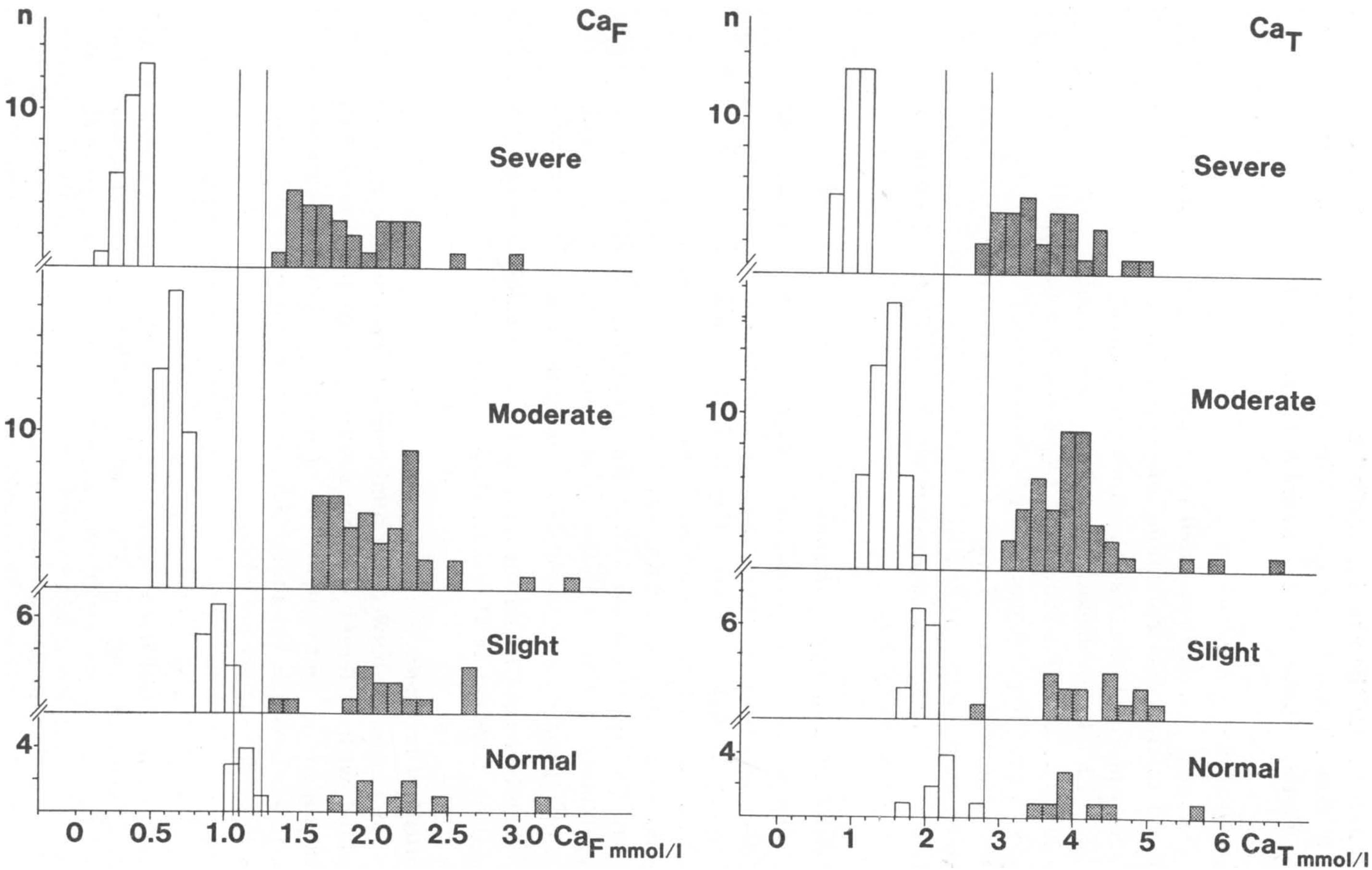


Figure 1 A and B.

The distribution of serum ionized (Ca_F) and total calcium (Ca_T) concentrations (mmol/l) in 97 cows with clinical signs of parturient paresis before (open bars) and after (closed bars) treatment with 8 g of calcium in the respective groups: normocalcemic (n = 8), slight (n = 15), moderate (n = 43) and severe hypocalcemia (n = 31). The reference range for both Ca_F and Ca_T are indicated (——).

estimated from the running control serum. Mean ± s was 2.45 ± 0.036 mmol/l and CV = 1.5 %. The reference range was 2.19—2.83 mmol/l (mean ± 2 s) (*Kvart & Larsson* 1978).

Classification of hypocalcemia

The material was classified in groups according to the serum ionized calcium concentration before treatment:

Normocalcemia: Ca_F 1.06—1.26 mmol/l

Slight hypocalcemia: Ca_F 0.80—1.05 mmol/l

Moderate hypocalcemia: Ca_F 0.50—0.79 mmol/l

Severe hypocalcemia: $Ca_F < 0.50$ mmol/l

where the lower cut off values for Slight and Moderate hypocalcemia were arbitrarily set to the lower reference limit (1.06 mmol/l) minus approximately 5 and 11 standard deviations, respectively.

Classification of hypercalcemia

The degree of hypercalcemia after treatment was also described according to serum ionized calcium concentrations:

Slight hypercalcemia: Ca_F 1.27—1.50 mmol/l

Moderate hypercalcemia: Ca_F 1.51—1.80 mmol/l

Severe hypercalcemia: $Ca_F > 1.80$ mmol/l

where the upper cut off values for Slight and Moderate hypercalcemia were set to the upper reference limit (1.26 mmol/l) plus approximately 5 and 11 standard deviations, respectively.

The increases in serum total and ionized calcium concentrations are denoted as ΔCa_T and ΔCa_F (mmol/l).

Statistical methods

Comparisons between serum calcium contrations in cows treated with different calcium solutions were performed with Student's t-test and comparisons of the therapeutical results was performed with a chi-square test according to principles given by *Snedecor & Cochran* (1967).

Figure 2 A and B.
The regression of total serum calcium on serum ionized calcium for the whole material before (A) and after (B) treatment with 8 g of calcium. Regression line corresponds to the equations: $Ca_T = 1.734 \times Ca_F + 0.303$, correlation coefficient 0.973 (Figure A) and $Ca_T = 1.250 \times Ca_F + 1.184$, correlation coefficient 0.770 (Figure B). The 95 % confidence limits for Ca_T are indicated at the Ca_F limits between the groups in the classification of hypo- and hypercalcemia.

Figure 2A

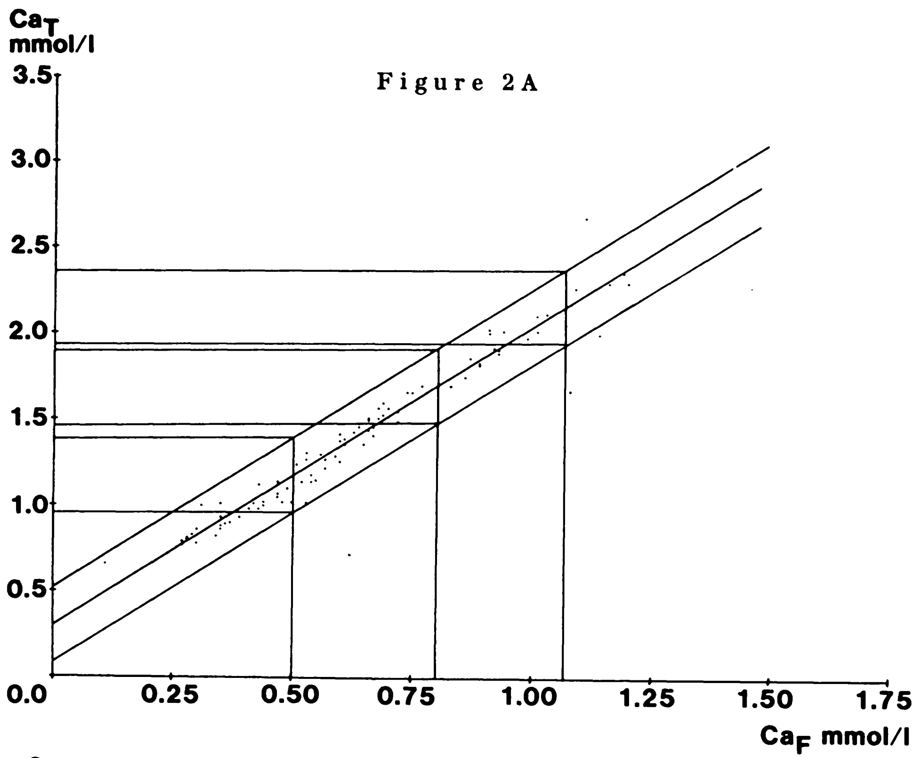
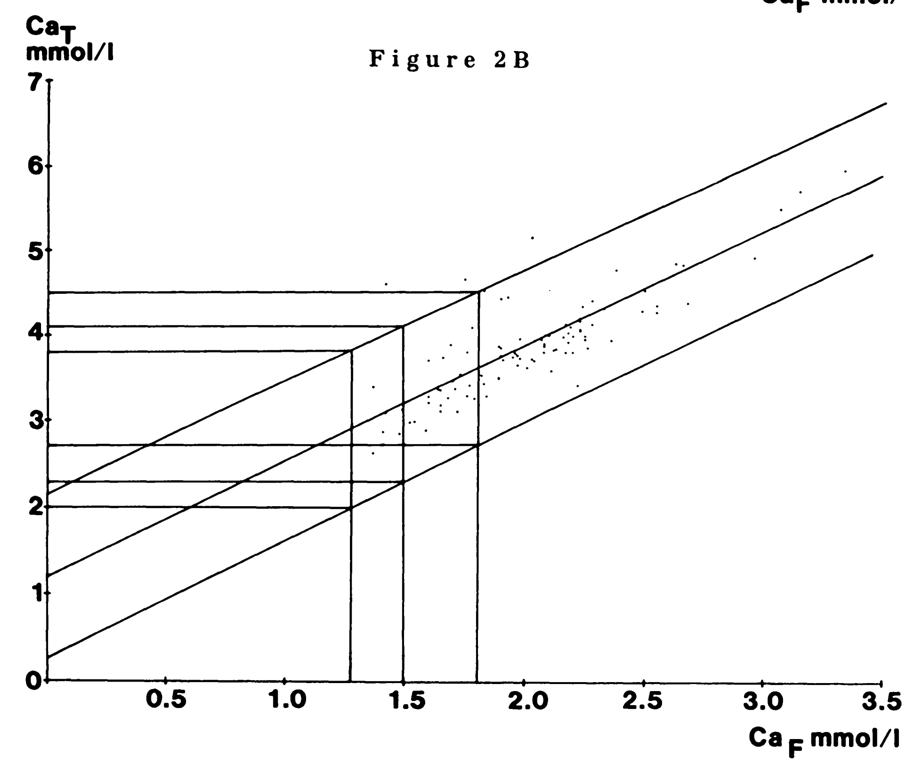


Figure 2B



RESULTS

The distributions of serum ionized and total calcium concentrations before treatment can be seen in Fig. 1. The correlation between Ca_F and Ca_T is shown in Fig. 2. Ninety-five % confidence limits for Ca_T at the limits between groups of cows classified by Ca_F values are given in Table 2.

Table 2. Ninety-five % confidence limits for Ca_T at the limits for hypo- and hypercalcemic groups classified by Ca_F values (cf. Methods).

Ca_F mmol/l	Ca_T mmol/l Mean	Confidence limits	
Hypocalcemia			
0.50	1.17	0.96	1.38
0.80	1.69	1.48	1.90
1.06	2.14	1.93	2.35
Hypercalcemia			
1.27	2.90	2.00	3.80
1.50	3.21	2.31	4.10
1.80	3.61	2.73	4.50

The distributions of maximum calcium concentrations after treatment regardless of administered drug, are seen in Fig. 1. Sixty-seven % (65/97) of all cows reached serum ionized calcium concentrations within the severe hypercalcemia range ($Ca_F > 1.80$ mmol/l) and 61 % (14/23) of the normocalcemia and slight hypocalcemia groups reached serum ionized calcium concentrations > 2.00 mmol/l, which is more tran 15 standard deviations above the upper reference limit (Fig. 1).

The increase of serum ionized calcium (ΔCa_F) was significantly greater ($t = 2.9$, $P < 0.01$, $DF = 20$) for calcium chloride ($\Delta Ca_F = 1.49 \pm 0.25$ mmol/l, mean ± s) than for calcium borogluconate ($\Delta Ca_F = 1.15 \pm 0.19$ mmol/l, mean ± s). For total calcium (ΔCa_T) no such difference was noted (ΔCa_T calcium chloride $= 2.41 \pm 0.78$ mmol/l, (mean ± s) and ΔCa_T calcium borogluconate $= 2.97 \pm 0.94$ mmol/l, (mean ± s). Both post-treatment Ca_F and Ca_T were negatively correlated to the infusion time. Although statistically significant ($r^2 = 0.067$ and 0.065, $DF = 80$, $P < 0.05$), most of the variation was due to other factors than the infusion time. ΔCa_F and ΔCa_T were not correlated to the infusion time ($r^2 = 0.010$ and 0.009, respectively), which should be explained by a higher ΔCa for those cows which initially had a low value (Fig. 1).

The therapeutic result with the different drugs can be seen in Table 3. There was no significant difference between the calcium preparations with respect to frequency of recovery.

Table 3. Therapeutical result with the different calcium solutions with respect to frequency of recovery. Two of the p.p. cows were treated with unknown calcium solutions.

Calcium preparation	Number of cows treated	Number of cows recovering after 1 treatment	%	Number of cows recovering after 2 treatments	%	Number of cows recovering after 3 treatments	%	Number of cows that did not recover	%
Calcium chloride	11	9	81.8	2	18.2				
Calcium boro-gluconate	15	10	66.7	5	33.3				
Cadexil	33	24	72.7	5	15.2	3	9.1	1	3.0
Novacalc	36	23	63.9	10	27.8	2	5.6	1	2.8

DISCUSSION

Ionized calcium is the physiologically active fraction (*McLean & Hastings* 1934, *Toffaletti et al.* (1980) and thus the ideal calcium fraction to be measured for assessment of disorders in calcium metabolism (*Ladenson* 1981). Furthermore, serum ionized calcium was recently reported of special value in diseases characterized by acute changes in calcium homeostasis (*Bruun* 1980).

Correction of the commonly available serum total calcium with respect to albumin may improve the situation when the latter deviates from normal (*Larsson & Öhman* 1979, *Editorial* 1979). Numerous correction factors are available but *Ladenson et al.* (1980) reported the failure of 13 different published methods to reliably predict serum ionized calcium. Thus the most accurate way to determine the physiologically active calcium fraction seem to be a direct measurement of ionized calcium.

In this study we devised a classification of the disturbance in serum calcium in cows with parturient paresis in terms of serum ionized calcium concentrations. This calcium fraction is seldom used in this way although *Hallgren et al.* (1959) classified their paretic cows into 3 groups on basis of clinical signs and reported decreasing Ca_F with progressive severity. Most investigations on parturient paresis use serum total calcium concentrations for classification with $Ca_T < 2.00$ mmol/l as cut off level and for pronounced hypocalcemia in p.p. cows $Ca_T < 1.50$ mmol/l has been used (*Jonsgaard* 1972, *Jönsson & Pehrsson* 1975). Although we choose arbitrary limits for our classification it is apparent that these 2 Ca_T levels correspond well with the lower confidence limits for serum total calcium in the slight and moderate hypocalcemia groups, respectively (see Table 2).

The degree of hypocalcemia in parturient paresis assayed as Ca_F was reported by *Sjollema & Seekles* (1930) to be 0.11 mmol/l. *Boogaardt* (1954), however, found an average concentration of 0.62 mmol/l and *Hallgren et al.* (1959) reported serum ionized calcium concentrations of 0.50 ± 0.03 mmol/l (mean $\pm$ s) when studying 90 cows with parturient paresis with the murexide method. One of the first flow-through electrodes for Ca_F assay (Orion 98-20) was used by *Blum et al.* (1973) who reported a mean of 0.44 mmol/l for 9 cows with parturient paresis. Our paretic cows had serum ionized calcium concentrations of 0.64 ± 0.25 mmol/l (mean $\pm$ s). Differences in material and methods may explain the discrepancy between these results.

This study underlines the difficulty in predicting serum ionized calcium from serum total calcium concentrations (Fig. 2). Here one can see that $Ca_T = 1.02$ mmol/l could mean $Ca_F = 0.53$ or 0.31 mmol/l. A subnormal serum total calcium of 2.00 mmol/l corresponds similarily to Ca_F 0.92 or 1.14 mmol/l. This discrepancy between Ca_T and Ca_F concentrations is in accordance with an earlier report for normal cows (*Kvart & Larsson* 1978) and for humans (*Larsson & Öhman* 1979, *Ladenson* 1978, 1981). Note the greater scattering in the Ca_F/Ca_T plot (Fig. 2) after treatment than before. Obviously, this is caused by the rapid change induced by the therapy but also influenced by differences in time for sampling.

In earlier reports on p.p. in the cow a certain number of the cows have been normocalcemic judged from Ca_T concentrations (*Trainin et al.* 1972: 23.6 %, *Ødegaard & Øverby* 1973: 15 %;

and *Mullen et al.* 1975: 3.7 %). We found 5 cows (5.2 %) with normal Ca_T and 8 (8.3 %) with normal Ca_F. In these and other studies all reported normocalccmic cases were clinically judged as parturient paretic cows. This indicates that other factors than calcium i.e. phosphate might influence the clinical picture. The frequency of discrepancies might be explained by differences in material and by the fact that p.p. cases well could be normocalcemic judged with serum total calcium determination, the most commonly used method for calcium assay, although the serum ionized calcium concentration was low. Unfortunately little information is available on the latter serum calcium fraction especially in most large clinical trials.

There are few reports in the literature establishing the maximum Ca_F concentrations after calcium infusion in parturient cows, a level which is of interest because of the risk for fatal cardiac arrythmias. *Blum et al.* (1972) reported a Ca_F maximum of about 1.88 mmol/l in 1 of the paretic cows and *Littledike et al.* (1976) in an experimental study on calves reported arrythmias to occur at a $Ca_F < 2.30$ mmol/l. In our study on clinical cases of parturient paretic cows arrythmias were found already during hypocalcemia as well as during the normo- and hypercalcemic phases of the calcium infusion. This indicates the complex background for development of cardiac disturbances. A detailed study on the type and frequency of arrythmias in 27 cows in this investigation will be published separately. Calcium chloride produces significantly higher serum ionized calcum maximum concentrations than calcium borogluconate when 8 g of calcium was infused intravenously for 8 min. In an experimental study *Hapke* (1974) reported an equally large increase in serum ionized calcium after treatment with calcium chloride and calcium borogluconate when using doses less than 20 mg/kg (i.e. 10 g calcium for a 500 kg cow). However, when *Hapke* increased the calcium dose above therapeutical levels calcium chloride gave a substantially higher ionized calcium concentration than equal amounts of calcium borogluconate.

Marr et al. (1955) reported a serum total calcium increase in a satisfactory and an unsatisfactory responding group of parturient paretic cows treated with calcium borogluconate to a maximum mean concentration of 2.44 mmol/l and 3.64 mmol/l in the respective groups. These Ca_T concentrations were lower than those obtained in most of our cows after treatment (see

Fig. 1). These differences might well be accounted for by differences in body size, time of blood sampling, administered calcium dose or differences in infusion time. In clinical practice calcium is commonly infused not faster than 1 g/min, i.e. 8 g of calcium should not be administered faster than 8 min. This study indicated that infusion time, in the interval 5—35 min, had only marginal effect on the maximum calcium concentration. A preliminary study on treatment of p.p. with an individual calcium dose after judgement of the severity of hypocalcemia indicates that lower maximum calcium concentrations are obtained with this treatment, which might reduce the risk for complications (*Kvart et al.*, in preparation). Although we found no significant difference in therapeutical results between different calcium solutions, clinical experience speaks in favour of different therapeutic effects with different calcium preparations (*Jönsson & Pehrsson* 1975). These observations emphasize the need of more complete elucidation of the therapeutical effects in treatment of parturient paresis. The high frequency of severe hypercalcemia after the treatment with 8 g of calcium might also question this type of routine therapy for all cows with parturient paresis.

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SAMMANFATTNING

Puerperal pares hos ko. Joniserat kalcium i serum före och efter behandling med olika kalciumlösningar — klassificering av olika grader av hypo- och hyperkalcemi.

Hos 97 kor (87 SRB och 10 SLB) med puerperal pares bestämdes serumkoncentrationen av joniserat kalcium (Ca_F). Därefter indelades materialet i olika grupper efter graden av hypokalcemi:

		Antal kor
Normokalcemi	Ca_F 1.06—1.26	8
Lindrig hypokalcemi	Ca_F 0.80—1.05	15
Måttlig hypokalcemi	Ca_F 0.50—0.79	43
Grav hypokalcemi	$Ca_F < 0.50$	31

Samtliga kor behandlades med intravenös infusion av 8 eller 8.3 g kalcium vilket resulterade i olika grad av hyperkalcemi. Hos 65 (67 %) av korna noterades grav hyperkalcemi efter behandling ($Ca_F > 1.80$ mmol/l), vilket kan ifrågasätta lämpligheten av standardiserad behandling för kor med puerperal pares. Den utförda studien antyder ett behov av ett kliniskt test för bedömning av graden av hypokalcemi omedelbart före behandling samt visar även svårigheten att förutsäga joniserat kalcium i serum från totalkalciumbestämning.

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