Acta vet. scand. 1975, *16*, 63—75.

From the Department of Medicine II, Royal Veterinary College,
Stockholm, Sweden.

LIPID MOBILIZATION IN PARTURIENT COWS*

By

J. Luthman and *J. Persson*

LUTHMAN, J. & J. PERSSON: *Lipid mobilization in parturient cows*. Acta vet. scand. 1975, *16*, 63—75. — The blood levels of calcium, inorganic phosphorus, magnesium, glucose and NEFA were studied in cows at the time around partus. Eight of 16 cows developed hypocalcaemic paresis. Besides hypocalcaemia the paretic cows showed lower levels of inorganic phosphorus and higher levels of glucose and NEFA than non-paretic cows 24 hrs. post partum. It is known that lipolysis is associated with uptake of calcium in adipose tissue. The calcium content in perirenal adipose tissue was however lower in paretic cows than in non-paretic parturient cows and lactating cows slaughtered 3—5 months after calving. The calcium content in omental adipose tissue was about the same in all 3 groups. Despite increased lipolysis the calcium content in adipose tissue is thus not increased in cows suffering from parturient paresis.

adipose tissue; calcium; lipolysis; non-esterified fatty acids.

It is now well established that calcium ions are of importance for the activation of various cell systems (*Rasmussen* 1970). Lipolysis is one of the processes where the role of calcium has been studied. *Efendic et al.* (1970) reported that lipolytic agents showed reduced effect in vitro when calcium was omitted from the incubation medium. Several authors have observed that lipolysis is associated with uptake of calcium in adipose tissue (*Alm et al.* 1970, *Storck & Björntorp* 1971, *Werner & Löw* 1973).

Several years ago *Friesen* (1964) observed that ACTH-induced lipolysis in rabbits was followed by hypocalcaemia. As the lowest serum calcium values occurred in the animals which showed the highest levels of non-esterified fatty acids (NEFA), it was suggested that the 2 phenomena in some way were related. ACTH-

* Supported by the Swedish Council for Forestry and Agricultural Research.

induced hypocalcaemia in rabbits was later studied by *Akgün & Rudman* (1969). They found that excessive lypolysis was associated with a remarkable uptake of calcium in adipose tissue. When NEFA reached levels of about 3 meq./l, the calcium content in adipose tissue was increased up to 1100 %. Their calculations showed that the calcium uptake in adipose tissue was sufficient to explain hypocalcaemia. *Lis et al.* (1972) obtained similar results in rabbits after administration of β-LPH, a lipolytic substance isolated from sheep pituitary glands.

The hypocalcaemic effect of lipolytic hormones has also been studied in sheep. *Mosely & Axford* (1971) reported that adrenaline-induced lipolysis in sheep was associated with hypocalcaemia and increased calcium content in adipose tissue. The decrease in serum calcium was well correlated to the increase in NEFA and to the calcium increase in adipose tissue. *Luthman & Holtenius* (1972) and *Luthman et al.* (1972) found that norepinephrine caused hypocalcaemia in sheep. The fall in serum calcium was correlated to the increase in NEFA. Hypocalcaemia was reduced when the lipolytic effect of norepinephrine was reduced by nicotinic acid.

Parturient cows show increased lipid mobilization (*Flatlandsmo* 1971). *Luthman & Jonson* (1972) found a significant negative correlation between NEFA and serum calcium in cows suffering from parturient paresis. The aim of the present investigation was therefore to study if the increased lipid mobilization in cows suffering from parturient paresis was associated with changes in the calcium content of adipose tissue.

MATERIAL AND METHODS

Sixteen cows of the Swedish Red and White breed (SRB) were used in the study. The age of the animals varied from 7 to 10 years. All cows had suffered from parturient paresis at least once previously. The cows were brought to the institute about 3 weeks before expected calving. Blood was sampled daily from about 6—7 days before calving, when signs of calving occurred, samples were taken with frequent intervals. Serum and plasma were separated as soon as possible and stored frozen until analyzed.

Fourteen of the cows were slaughtered after calving. The cows which showed hypocalcaemic paresis were slaughtered at a time when it was considered they otherwise should have died. The non-paretic cows were slaughtered 24—48 hrs. post partum. Samples from the perirenal and omental adipose tissue were taken from each cow. For comparative purposes adipose tissue samples were also taken from 7 non-pregnant lactating cows, slaughtered 3—5 months after calving.

Plasma was used for determination of calcium and NEFA, while magnesium and inorganic phosphorus was determined on serum.

Calcium was analyzed according to *Skerry* (1965) and NEFA according to *Dole* (1956). Magnesium and inorganic phosphorus was determined by commercial reagents (Merckotest Magnesium, E. Merck, Darmstadt, Germany and Sigma Kit 670, Sigma Chemical Company, St. Louis, USA).

Glucose was determined on whole blood according to the glucose oxidase method (reagents from AB Kabi, Stockholm, Sweden).

Adipose tissue calcium was analyzed according to the procedure described by *Akgün & Rudman* (1969).

Conventional statistical methods were used (Student's t-test for paired and unpaired data).

RESULTS

Parturient paresis developed in 8 cows. One cow was paretic at parturition, the other cows showed signs of paresis 6—12 hrs. post partum.

The changes in the measured parameters at the time around partus are shown in Figs. 1—3. Table 1 shows the mean values in the paretic and the non-paretic cows. Calcium was lower at parturition than on the previous day in both groups, but remained unchanged 24 hrs. post partum in the non-paretic cows. The lowest level obtained in a non-paretic cow was 6.2 mg/100 ml, 6 hrs. later the level had increased to 7.2 mg/100 ml.

Inorganic phosphorus decreased at parturition in both groups. The paretic cows showed significantly lower levels 24 hrs. post partum.

Serum magnesium increased at parturition, but there was no significant difference between the groups.

NEFA and blood glucose increased markedly at parturition.

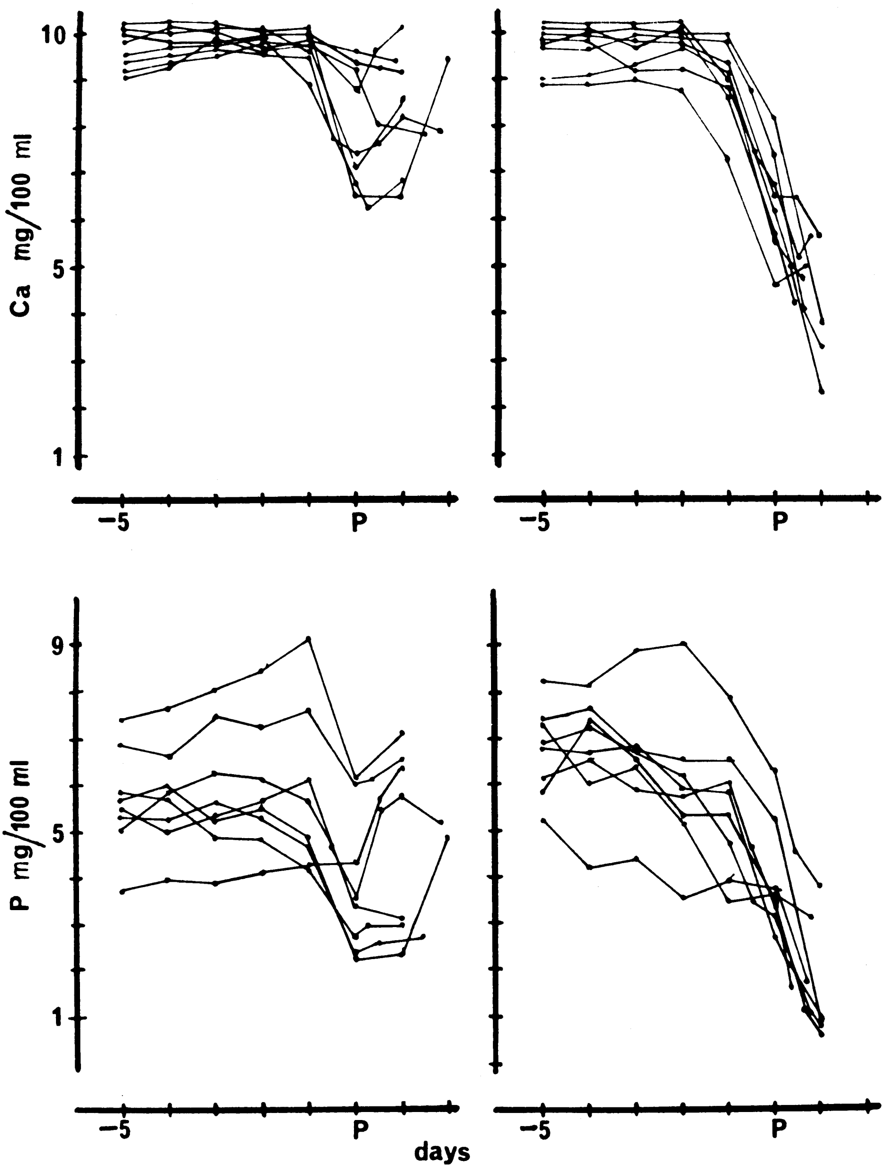

Figure 1. Changes in plasma calcium and serum inorganic phosphorus in non-paretic (left) and paretic cows (right) at the time around partus (P).

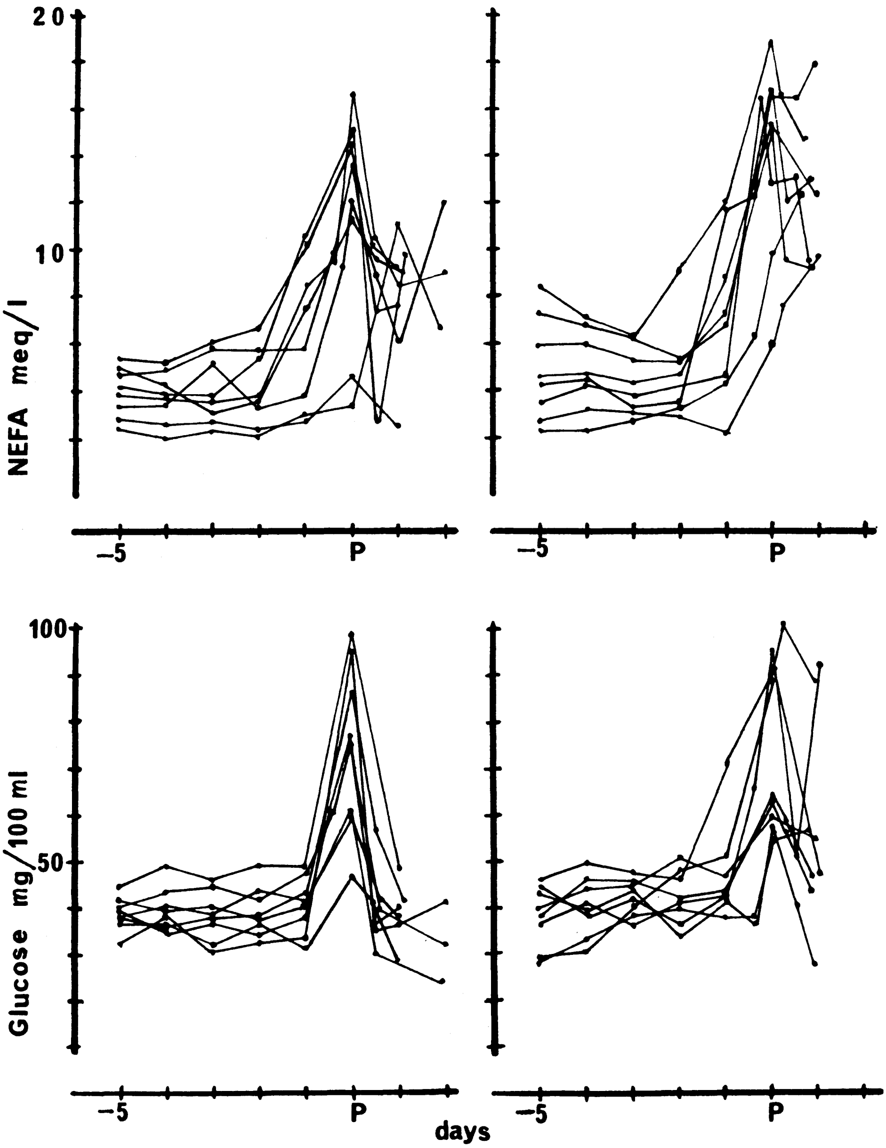

Figure 2. Changes in blood glucose and NEFA in non-paretic (left) and paretic cows (right) at the time around partus (P).

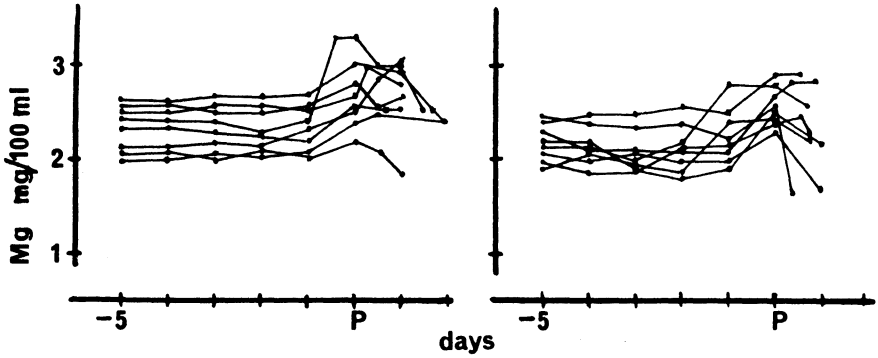

Figure 3. Changes in serum magnesium in non-paretic (left) and paretic cows (right) at the time around partus (P).

Table 1. Blood levels of calcium, inorganic phosphorus, magnesium, glucose and NEFA at the time around partus. Mean ± s.

	A 5 days ante partem	B 1 day ante partem	C partus	D 24 hrs. post partum	Comparisons within the group A—B	 B—C	 C—D
			Calcium (mg/100 ml)				
Non-paretic (8)	9.51±0.46	9.63±0.33	8.15±1.25	8.33±1.30	n.s.	**	n.s.
Paretic (8)	9.73±0.34	9.18±0.91	6.38±1.15	4.41±1.22	n.s.	***	*
Comparison between the groups	n.s.	n.s.	*	***			
			Inorganic phosphorus (mg/100 ml)				
Non-paretic (8)	5.96±1.20	5.80±1.80	3.85±1.47	4.49±1.91	n.s.	**	*
Paretic (8)	6.64±1.28	5.41±1.50	3.90±1.22	1.65±1.20	**	**	***
Comparison between the groups	n.s.	n.s.	n.s.	**			
			Magnesium (mg/100 ml)				
Non-paretic (8)	2.36±0.26	2.35±0.27	2.66±0.34	2.63±0.41	n.s.	**	n.s.
Paretic (8)	2.20±0.29	2.26±0.29	2.53±0.22	2.35±0.40	n.s.	*	n.s.
Comparison between the groups	n.s.	n.s.	n.s.	n.s.			
			NEFA (meq./l)				
Non-paretic (8)	0.37±0.10	0.60±0.33	1.39±0.41	0.78±0.26	*	**	n.s.
Paretic (8)	0.47±0.24	0.70±0.35	1.14±0.51	1.21±0.33	n.s.	***	n.s.
Comparison between the groups	n.s.	n.s.	n.s.	*			
			Glucose (mg/100 ml)				
Non-paretic (8)	38±4	40±6	76±16	38±6	n.s.	***	***
Paretic (8)	39±6	47±11	72±17	58±22	*	**	*
Comparison between the groups	n.s.	n.s.	n.s.	*			

* $0.05 > P > 0.01$ ** $0.01 > P > 0.001$ *** $P < 0.001$

Table 2. Correlations of calcium, inorganic phosphorus, magnesium, glucose and NEFA in parturient cows. Samples obtained > 3 hrs. post partum. Ca < 8 mg/100 ml. (n = 18)

Ca vs. P	r = 0.868***
Ca vs. Mg	r = 0.428
Ca vs. glucose	r = —0.268
Ca vs. NEFA	r = —0.684**
P vs. Mg	r = 0.525*
P vs. glucose	r = —0.080
P vs. NEFA	r = —0.615**
Mg vs. glucose	r = —0.099
Mg vs. NEFA	r = —0.454
Glucose vs. NEFA	r = 0.624**

* $0.05 > P > 0.01$
** $0.01 > P > 0.001$
*** $P < 0.001$

Blood glucose varied widely in the paretic cows, the lowest value obtained was 25 mg/100 ml and the highest 101 mg/100 ml. The mean values of both glucose and NEFA were higher in the paretic than in the non-paretic cows 24 hrs. post partum.

There was no significant correlation between the plasma levels of NEFA and calcium at parturition ($r = -0.327$, $P > 0.05$).

Table 2 shows the correlations between the measured parameters in all cows with plasma calcium levels below 8 mg/100 ml. Samples taken before and less than 3 hrs. after parturition were not included. Calcium and inorganic phosphorus were significantly correlated, and there was a negative correlation between calcium and NEFA and between inorganic phosphorus and NEFA. An almost significant positive correlation existed between inorganic phosphorus and magnesium.

The NEFA mean in the lactating cows, slaughtered 3—5 months post partum, was 0.30 ± 0.06 meq./l, calcium was 9.80 ± 0.21 and inorganic phosphorus 6.50 ± 0.43 mg/100 ml.

Table 3 shows the calcium content in adipose tissue. Perirenal adipose tissue from the paretic cows contained less calcium than perirenal tissue from the non-paretic cows and from lactating cows slaughtered 3—5 months after calving. The calcium content in omental adipose tissue was about the same in the 3 groups.

Table 3. Calcium content in adipose tissue (μg/g wet tissue) of parturient and non-parturient cows. Mean ± s.

	Parturient non-paretic (n = 7)	Parturient paretic (n = 7)	Non-parturient (n = 7)
Perirenal adipose tissue	11.76±7.65	5.17±1.07	12.77±6.47
	0.05 > P > 0.01		0.01 > P > 0.001
Omental adipose tissue	13.08±7.56	11.86±9.77	12.02±4.00

DISCUSSION

Parturient cows with calcium levels below 8 mg/100 ml are in general considered as hypocalcaemic (*Mayer et al.* 1966, *Jönson & Pehrson* 1969). *Mayer et al.* reported that severe hypocalcaemia without signs of paresis sometimes occurs. It is obvious from the work of *Mayer et al.* and from that of *Willoughby et al.* (1970) that no critical plasma calcium level exists below which paresis occurs. In the present study 3 of 8 non-paretic cows showed calcium levels below 8 mg/100 ml within the first 24 hrs. post partum. The causes to the development of paresis have been a matter for discussion. *Bowen et al.* (1970) presented evidence for the hypothesis that hypocalcaemia causes a block of the neuromuscular transmission. *Rahamimoff* (1970) stated that the nerve terminal is very sensitive to low environmental calcium levels, and that calcium ions thus are necessary for the release of acethylcholine. *Kowalczyk & Mayer* (1972) found that the cellular concentration of calcium in muscles from paretic cows was sufficient for muscle contraction. They also found that muscles from paretic cows showed increased sodium and decreased potassium concentration. On the basis of this finding they suggested that the inexcitability of the muscles may be due to changes in the transmembrane potential of the muscle cells, caused by the altered electrolyte status.

As seen from Table 1 plasma inorganic phosphorus decreased at parturition in both groups of cows. The paretic cows showed a significantly lower level 24 hrs. post partum. Similar findings have been reported by several authors e. g. *Carlström* (1961).

The magnesium level increased at parturition, but there were no significant differences between the groups. Varying magnesium levels have been obtained in paretic cows (*Kronfeld & Ram-*

berg 1970). *Hallgren et al.* (1959) found significantly elevated magnesium levels in paretic cows, while *Carlström* obtained similar values for paretic as well as non-paretic cows.

The highest levels of glucose and NEFA occurred at parturition. It seemed as the increase in both glucose and NEFA was related to the intensity of labour. The cows in which calving was complicated by large foetuses showed the highest levels. The elevation of glucose and NEFA at parturition may thus be taken as signs of a general stress reaction. Both glucose and NEFA varied within a wide range in the paretic cows. The mean levels were however almost significantly higher than in the non-paretic cows 24 hrs. post partum. The NEFA mean obtained in the paretic cows, 1.21 $\pm$ 0.33 meq./l, is almost identical with that reported earlier (*Luthman & Jonson* 1972). *Littledike et al.* (1968) found extremely high plasma glucose levels in paretic cows, values up to 160 mg/100 ml were observed. The glucose changes obtained in the present study agree well with the results of *Blum et al.* (1972).

Table 2 shows the correlations between the measured parameters. All samples taken at parturition and within the first 3 hrs. post partum are not included, since the intention was to study these correlations in uncomplicated hypocalcaemia. As discussed above the increase in blood glucose and NEFA at parturition seemed to be related to stress rather than to the degree of hypocalcaemia. It is known that several blood parameters, not only glucose and NEFA, change under stressful conditions. *Persson & Luthman* (1974a) found e. g. that norepinephrine induced a significant reduction of plasma magnesium in sheep.

As seen from Table 2 there was a significant positive correlation between calcium and inorganic phosphorus and a significant negative correlation between calcium and NEFA. Glucose and NEFA were positively correlated as was inorganic phosphorus and magnesium.

Littledike et al. reported that hypocalcaemic cows showed reduced plasma insulin levels and were unable to increase insulin secretion in response to glucose infusions. *Blum et al.* (1973) made similar observations. It was therefore concluded that hyperglycaemia in paretic cows was due to hypoinsulinaemia. Increased lipid mobilization could then be expected in light of the reduced glucose utilization. Hypocalcaemic cows show increased parathyroid activity and increased blood levels of parathyroid hormone (*Mayer* 1970). It is therefore generally considered that the reduction of inorganic phosphorus in paretic cows is in part due to parathyroid hormone-induced renal phosphate excretion. There is now evidence for the hypothesis that also the increased blood glucose and NEFA levels may be caused by parathyroid hormone, since it was recently found that parathyroid hormone shows a pronounced lipolytic effect and a moderate hyperglycaemic effect in ruminants (*Persson & Luthman* 1974 b).

Blum et al. (1972) reported a significant negative correlation between magnesium and inorganic phosphorus in hypocalcaemic cows, similar results were obtained in hypocalcaemic sheep by *Jonson et al.* (1973). In the present study there was a positive correlation between magnesium and inorganic phosphorus. These different results are difficult to explain.

The increased lipid mobilization at the time around partus was not associated with increased calcium content in adipose tissue. The non-paretic cows showed higher NEFA levels than the non-pregnant lactating cows, but the calcium content in adipose tissue was about the same. In all the in vivo experiments where calcium was taken up in remarkable amounts in adipose tissue, NEFA has reached very high levels, 3 meq./l or even more. The NEFA mean in the non-paretic cows was 0.78 ± 0.26 meq./l. It is possible that lipolysis was not increased to an extent sufficient to cause a measurable shift of calcium into adipose tissue. NEFA was 1.21 ± 0.33 meq./l in the paretic cows at slaughter, but the calcium content in the perirenal adipose tissue was lower than in the non-paretic cows. It is obvious that hypocalcaemia is associated with a loss of tissue calcium. *Kowalczyk & Mayer* found that the calcium content was low in muscles from hypocalcaemic cows. The calcium concentration was however considered to be sufficient for muscle contraction. The different calcium content in perirenal and omental adipose tissue may perhaps be due to different metabolic activity of the tissues.

Calcium uptake in adipose tissue to such an extent that hypocalcaemia occurs has hitherto only been observed in experimental conditions. The significance of this mechanism in calcium homeostasis still remains obscure.

REFERENCES

Akgün, S. & D. Rudman: Relationships between mobilization of free fatty acids from adipose tissue and the concentration of calcium in the extracellular fluid and in the tissue. Endocrinology 1969, *84*, 926—930.

Alm, B., S. Efendic & H. Löw: Effect of lipolytic and antilipolytic agents on the uptake of ^{47}calcium into rat adipose tissue in vitro. Horm. metab. Res. 1970, *2*, 142—146.

Blum, J. W., C. F. Ramberg, K. G. Johnson & D. S. Kronfeld: Calcium (ionized and total), magnesium, phosphorus, and glucose in plasma from parturient cows. Amer. J. vet. Res. 1972, *33*, 51—56.

Blum, J. W., R. B. Wilson & D. S. Kronfeld: Plasma insulin concentration in parturient cows. J. Dairy Sci. 1973, *56*, 459—464.

Bowen, J. H., D. M. Blackman & J. E. Heavner: Neuromuscular transmission and hypocalcemic paresis in the cow. Amer. J. vet. Res. 1970, *31*, 831—839.

Carlström, G.: Phosphorus, magnesium and pH in bovine blood serum normally, after parturition, and in parturient paresis. Acta vet. scand. 1961, *2*, 350—356.

Dole, V. P.: A relation between non-esterified fatty acid in plasma and the metabolism of glucose. J. clin. Invest. 1956, *35*, 150—154.

Efendic, S., B. Alm & H. Löw: Effects of Ca^{++} on lipolysis in human omental adipose tissue in vitro. Horm. metab. Res. 1970, *2*, 287—291.

Flatlandsmo, K.: Free glycerol in the plasma of cows. Acta vet. scand. 1971, *12*, 489—495.

Friesen, H.: Hypocalcemic effect of pituitary polypeptides in rabbits. Endocrinology 1964, *75*, 692—697.

Hallgren, W., G. Carlström & G. Jönson: Studies on parturient paresis. III. Determination of calcium ion concentration in bovine serum in different hypocalcaemic and pseudohypocalcaemic conditions. Nord. Vet.-Med. 1959, *11*, 217—249.

Jonson, G., J. Luthman, L. Möllerberg & J. Persson: Mineralutfodringen i fårbesättningar med kliniska fall av hypokalcemi. (The mineral feeding in sheep herds with clinical cases of hypocalcaemia). Nord. Vet.-Med. 1973, *25*, 97—103.

Jonson, G. & B. Pehrson: Studies on the downer syndrome in dairy cows. Zbl. Vet. Med. A, 1969, *16*, 757—784.

Kowalczyk, D. F. & G. P. Mayer: Cation concentration in skeletal muscle of paretic and nonparetic cows. Amer. J. vet. Res. 1972, *33*, 751—757.

Kronfeld, D. S. & C. F. Ramberg: Parturient paresis. *In* Bovine Medicine and Surgery. Gibbons, W. J., E. J. Catcott & F. J. Smithcors, eds. American Veterinary Publications Inc., Wheaton 1970.

Lis, M., C. Gilardeau & M. Chretien: Relationships between calcium transport and the lipolytic effect of sheep β-lipotropic hormone in adipose tissue. Acta endocr. (Kbh.) 1972, *69*, 507—516.

Littledike, E. T., D. A. Witzel & S. C. Whipp: Insulin: Evidence for inhibition of release in spontaneous hypocalcaemia. Proc. Soc. exp. Biol. (N.Y.) 1968, *129*, 135—139.

Luthman, J. & P. Holtenius: Norepinephrine-induced fatty liver and hypocalcemia in sheep. Acta vet. scand. 1972, *13*, 31—41.

Luthman, J. & G. Jonson: The relationship between serum calcium and plasma non-esterified fatty acids in normal and hypocalcemic cows and sheep. Acta vet. scand. 1972, *13*, 42—55.

Luthman, J., G. Jonson & J. Persson: Studies on norepinephrine-induced hypocalcemia in sheep before and after thyroidectomy. Acta vet. scand. 1972, *13*, 20—30.

Mayer, G. P.: The roles of parathyroid hormone and thyrocalcitonin in parturient paresis. *In* Parturient Hypocalcemia. Anderson, J. J. B., ed., Acad. Press, New York & London 1970.

Mayer, G. P., C. F. Ramberg & D. S. Kronfeld: Hypocalcemia without paresis in cows. J. Amer. vet. med. Ass. 1966, *149*, 402—405.

Mosely, G. & R. F. E. Axford: Redistribution of calcium in sheep induced by stress. Proc. Nutr. Soc. 1971, *30*, 58A.

Persson, J. & J. Luthman: The effect of insulin, glucose and catecholamines on some blood minerals in sheep. Acta vet. scand. 1974a, *15*, 519—532.

Persson, J. & J. Luthman: Some acute metabolic effects of parathyroid hormone in sheep. Acta vet. scand. 1974b, *15*, 381—397.

Rahamimoff, R.: Role of calcium ions in neuromuscular transmission. *In* Calcium and Cellular Function. Cuthbert, A. W., ed., MacMillan and Co. Ltd. London 1970.

Rasmussen, H.: Cell communication, calcium ions, and cyclic adenosine monophosphate. Science 1970, *170*, 404—412.

Skerry, D. W.: Di-(2hydro-phenyl-imino)-ethane: A new indicator for the EDTA titration of serum calcium. Clin. chim. Acta 1965, *12*, 593—597.

Storck, G. & P. Björntorp: Triglyceride hydrolysis and calcium accumulation in the rat epididymal fat pad exposed in vitro to norepinephrine and pancreatic lipase. Scand. J. Gastroent. 1971, *6*, 231—236.

Werner, S. & H. Löw: Stimulation of lipolysis and calcium accumulation by parathyroid hormone in rat adipose tissue in vitro after adrenalectomy and administration of high doses of cortisone acetate. Horm. metab. Res. 1973, *5*, 292—296.

Willoughby, R. A., D. G. Butler, J. F. Cote & R. A. Curtis: A study of clinical and meteorological data in parturient paresis in cattle. *In* Parturient Hypocalcemia. Anderson, J. J. B., ed., Acad. Press, New York & London 1970.

SAMMANFATTNING

Fettmobiliseringen hos kor vid kalvningen.

Förändringar i blodkoncentrationerna av kalcium, oorganisk fosfor, magnesium, glykos och NEFA studerades hos kor i anslutning till

kalvningen. Kor som drabbades av pares uppvisade förutom hypokalcemi och hypofosfatemi förhöjda värden för glykos och NEFA. Det är känt att kalciumupptaget i fettvävnaden ökar vid ökad lipolys. Trots att fettmobiliseringen var ökad hos pareskor, så var kalciuminnehållet i den perirenala fettvätnaden lägre än hos normala kor. Hypokalcemi kan förmodligen medföra en vävnadsförlust av kalcium som överstiger det upptag som sker i samband med ökad lipolys.

(Received June 6, 1974).

Reprints may be requested from: Jan Luthman, Department of Medicine II, Royal Veterinary College, S-104 05 Stockholm, Sweden.