Journal of Dairy Research (2004) **71** 316–321. © *Proprietors of Journal of Dairy Research 2004*
DOI: 10.1017/S0022029904000366 *Printed in the United Kingdom*

Changes of physicochemical indicators during mastitis and the effects of milk ejection on their sensitivity

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Received 29 August 2003 and accepted for publication 28 November 2003

We examined the relationship between physicochemical indicators and somatic cells in the milk of dairy cows during experimentally induced mastitis and their significance as indicators for use in controlling udder health. We were concerned particularly with the effect of alveolar milk ejection on the sensitivity of these indicators. In Expt 1, *Escherichia coli* lipopolysaccharide (*Esch. coli* LPS) was injected into the left rear quarter to induce an inflammatory reaction in one quarter in each of six cows. The contralateral control quarter was injected with a solution of NaCl (9 g/l). Nine milk samples were taken from both quarters until 60 h after injection. In Expt 2, repeated milk samples were taken every 20 s from one quarter during a 120-s teat stimulation in 20 cows with different somatic cell counts (SCC). Quarters were clustered for low (<5·0 log cells/ml), mid (5·0–5·7 log cells/ml) and high (>5·7 log cells/ml) SCC of the sample taken at t=0 s. Samples were analysed for SCC, electrical conductivity (EC) and Na^+ and Cl^- concentrations. During the experimental inflammation SCC, EC, Na^+ and Cl^- peaked at 12 h from LPS administration and values in treated quarters (T) at this time were elevated to 7900, 157, 501 and 169% of the values in untreated quarters, respectively. In Expt 2, SCC, EC, Na^+ and Cl^- in high SCC quarters were 2520, 121, 283 and 141% of low SCC quarters at the start of stimulation (t=0 s), respectively. Highly significant ($P<0·001$) differences in EC, Na^+ and Cl^- between high and low SCC quarters disappeared owing to the onset of alveolar milk ejection 100 s after the first contact with the teat. In conclusion, SCC in cows' milk provided the strongest amplitude in the case of an intramammary inflammation. EC, Na^+ or Cl^- were useful tools only if the measurements were performed in cisternal milk before the start of alveolar milk ejection.

Keywords: Electrical conductivity, ions in milk, SCC, milk ejection.

Electrical conductivity (EC) in milk has been discussed as an indicator of mammary inflammation for decades (Malcolm et al. 1942; Linzell & Peaker, 1975; Fernando et al. 1982). Results of experimental devices to measure EC were promising (Linzell & Peaker, 1975), although EC is influenced by parameters such as milking interval and milk composition (Wittkowski et al. 1979; Fernando et al. 1981; Woolford et al. 1998; Ontsouka et al. 2003). In-line systems were established to measure EC throughout milking (Gebre-Egziabher et al. 1979; Onyango et al. 1988). However, despite the larger pool of available data the sensitivity in detecting mastitis was not satisfactory for practical use (Maatje et al. 1992; Nielen et al. 1995).

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EC is determined by the ions dissolved in milk, mainly Na^+ and Cl^- (Linzell & Peaker, 1975; Wittkowski et al. 1979; Fernando et al. 1982). The ion concentration in normal milk is much lower than in blood serum. In the case of a mammary infection, the tight junctions lose integrity and the epithelium becomes leaky owing to neutrophil diapedesis and tissue damage by mastitis pathogens (Stelwagen et al. 1997; Nguyen & Neville, 1998). Consequently blood and milk components pass through the leaky epithelium.

The major proportion of milk, which in dairy cows is stored in the alveolar tissue, is available only after the start of milk ejection (Knight et al. 1994; Pfeilsticker et al. 1996). However, as much as 20% of the milk is stored in the cisternal compartment of the udder and is immediately available for milk removal before milk ejection occurs (Bruckmaier & Blum, 1998). Changes in EC in different

milk fractions within a specific milking event have been discussed (Linzell & Peaker, 1975; Wittkowski et al. 1979; Hamann & Gyodi, 2000; Ontsouka et al. 2003). However, the specific effect of alveolar milk ejection induced by teat stimulation without milk being removed, even by solely taking milk samples, has not been studied before.

Two experiments were conducted to study the sensitivity of EC, Na^+ and Cl^- measurements in detecting mastitis as compared with SCC. The hypothesis tested was that the expected exponential increase of SCC in response to an experimentally induced mastitis is accompanied by a clearly detectable increase of Na^+ and Cl^- concentrations and EC in the milk, which allows the use of these measures for the detection of mastitis. Most importantly, the influence of the occurrence of alveolar milk ejection on the ability of foremilk samples to detect udder health status via SCC, EC, and concentrations of Na^+ and Cl^- was investigated.

Materials and Methods

Animals and milking management

Cows were kept in loose housing and were milked in a milking parlour at 05.00 and 16.00. The diet was maize and grass silage, hay and concentrate given according to individual production levels. Cows were in their first to ninth lactation and were 50–206 d in milk. In Expt 1, Brown Swiss cows were used, whereas seven Red Holstein and thirteen Brown Swiss cows with a daily yield of 29·4±1·7 kg were used in Expt 2.

Experimental design

Expt 1. Experiments were conducted in six clinically healthy cows. Only rear quarters with a log SCC <5·2 and which cultured negative for mastitis pathogens in a sample taken 7 d before the experiment were accepted for the study. *Esch. coli* endotoxin (lipopolysaccharide, LPS; 100 μg) dissolved in 10 ml of NaCl (9 g/l) was injected intramammarily into the left rear quarter (T) after the morning milking. The right rear quarter (C) was injected with 10 ml of NaCl solution only. Cisternal milk samples of 50 ml were collected without any prior stimulation immediately before and at 3, 6, 9 h after LPS treatment and during succeeding milkings at 12, 24, 36, 48 and 60 h after LPS treatment.

Expt 2. Repeated milk sampling was performed during pre-stimulation in twenty cows. All teats were manually stimulated for 120 s. Milk samples (40 ml) were collected from the start until the end of stimulation at 20-s intervals from one quarter, while the other quarters were continuously stimulated. Thus, seven consecutive samples were taken. The udder was not touched before the start of stimulation and sampling. The procedure was repeated for three consecutive milkings in each cow.

Milk samples were analysed for SCC in the laboratory of the Milchprüfring Bayern e.V., 80336 München, Germany, using a MilkoScan 4500 analyser (Foss Electric, 3400 Hillerød, Denmark). Aliquots of each sample were frozen at –20 °C immediately after sampling for the determination of EC and Na^+ and Cl^- concentrations. EC was measured at 25 °C using the LDM electrode from WTW (LDM 130, Wissenschaftlich-Technische-Werkstaetten GmbH, 82362 Weilheim, Germany). Na^+ and Cl^- were determined in raw milk using the ion selective electrodes, models 9811 and 9617BN respectively (Orion Research, Beverly MA, USA).

Statistical analyses

Results are presented as means±SEM. Results were analysed using the repeated measures analysis of the MIXED procedure and tested for significance ($P<0·05$) using Least Significant-Difference Test (LSD) of the SAS (version 8.01) program package. For Expt 2, quarters were classified according to the SCC of the first sample obtained at t=0 s. Three clusters were used: SCC <5·0 log cells/ml (SCC I), 5·0–5·7 log cells/ml (SCC II) and >5·7 log cells/ml (SCC III). In Expt 1, the mixed model contained the treatment and the time of sampling as fixed effects and the animal was considered as a repeated factor. Likewise in Expt 2, the model included the time of sampling and the SCC cluster as fixed effects, and the animal entered the model as repeated factor.

Results

Expt 1

SCC before treatment was not significantly different between T and C quarters although a slight but insignificant rise of SCC was observed as compared with the sample taken 7 d before the experiment in some of the control quarters. Values of EC, Na^+ and Cl^- were low and did not differ between the quarters (Fig. 1). Within 3 h after LPS application, SCC, EC, Na^+ and Cl^- increased in T quarters. In C quarters, EC, Na^+ and Cl^- increased transiently owing to the administration of NaCl (9 g/l) solution and were numerically but not significantly higher at 3 h than at 0 h. Subsequently, SCC and Na^+ were significantly higher in T quarters than in C quarters between 6 and 24 h after administration of LPS and NaCl, respectively. EC and Cl^-, however, were significantly higher in T than in C quarters only at 9 and 12 h after LPS and NaCl treatments, respectively. SCC, EC, Na^+ and Cl^- peaked at 12 h after LPS administration. Their peak values in T quarters, expressed as linear values, were 7900, 157, 501 and 169% of C quarters, respectively. SCC remained elevated in T quarters until the end of the experiment (60 h), whereas all

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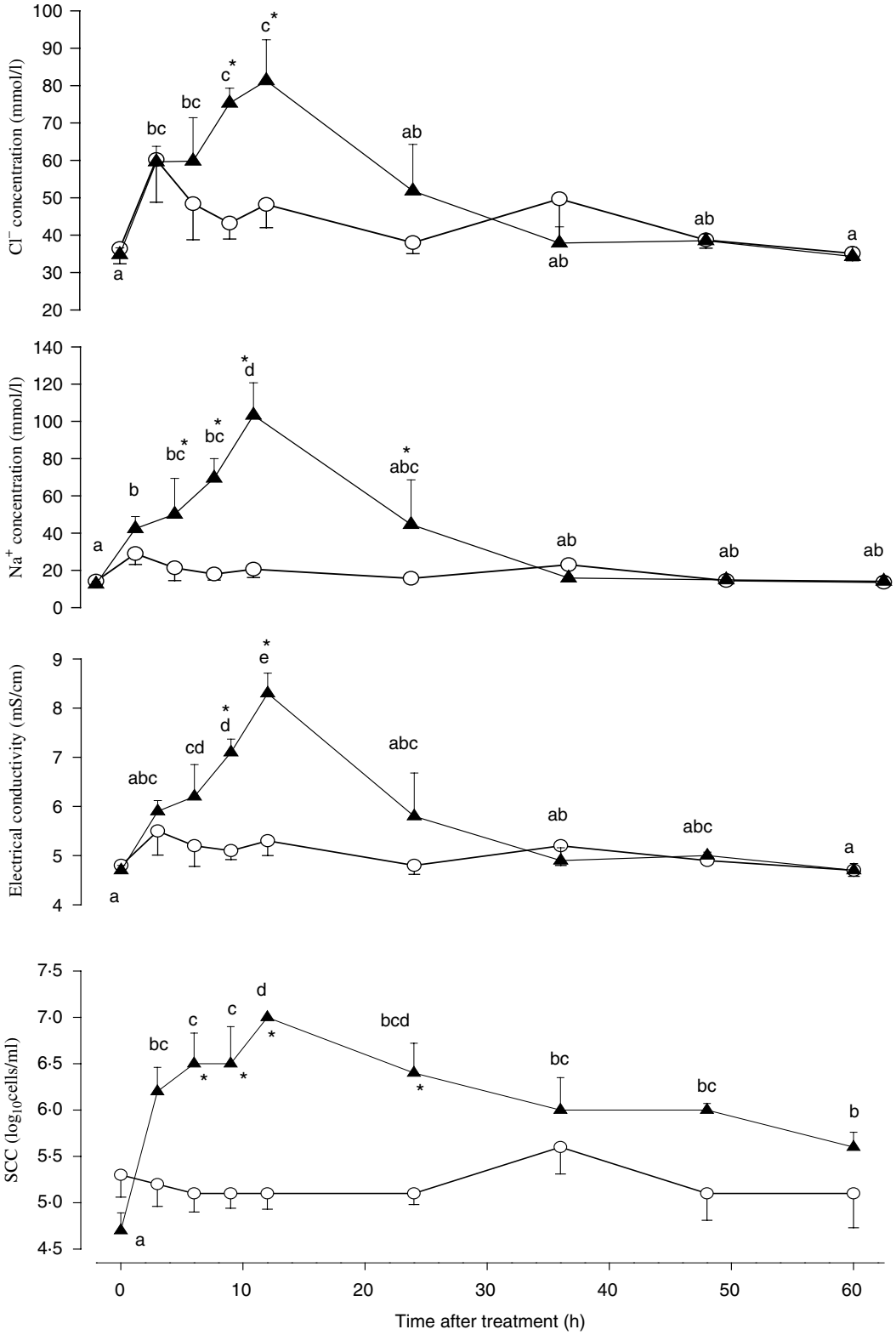

Fig. 1. SCC, EC, Na^+ and Cl^- concentrations in the milk of one treated (▲) and one control quarter (○) before and after application of 100 µg lipopolysaccharide (LPS) or NaCl control solutions, $n=6$ cows; $^{a, b, c, d}$ means without a common letter throughout the time span are different in treated quarters at corresponding times ($P<0{\cdot}05$); * indicates a significant ($P<0{\cdot}05$) difference between treated and control quarters.

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other parameters returned to pre-treatment levels within 24 h after LPS treatment.

Expt 2

Figure 2 illustrates the results obtained in Expt 2. In the first sample (t=0 s) mean SCC was 4·62±0·07, 5·34±0·04 and 6·00±0·08 log cells/ml in SCC I (n=23 samples), SCC II (n=14 samples) and SCC III (n=22 samples), respectively. In the first sample (t=0 s) mean SCC in SCC III quarters, expressed as a linear value, was 2520% of the results in SCC I quarters. SCC decreased within 120 s and was significantly lower at 100 s and 120 s than at the start of sampling (t=0 s) in SCC III and SCC I quarters. However, the SCC group differences between SCC I, SCC II and SCC III quarters remained significant throughout the 120-s sampling period.

From 0 to 60 s, EC in SCC III quarters was significantly higher than in SCC I and SCC II quarters, whereas EC did not differ significantly between SCC I and SCC II quarters. EC in SCC III quarters was 121% of that in SCC I quarters at t=0 s. A dramatic decrease of EC occurred between 40 and 100 s of sampling. In SCC III quarters, EC was significantly lower at t=80 s than at t=0 s. The difference of EC between SCC III and SCC II quarters was no longer significant from t=100 s, i.e., EC was similar in SCC III, SCC II and SCC I quarters 100 s after the start of sampling.

Na^+ was significantly higher in SCC III quarters than in SCC I throughout the 120-s sampling period, whereas for Cl^-, the significant difference between SCC I and SCC III quarters disappeared at t=100 s. At t=0 s, Na^+ and Cl^- concentrations in SCC III quarters were 283 and 141% of SCC I quarters, respectively. Na^+ and Cl^- did not differ significantly between SCC I and SCC II quarters throughout the sampling period. Despite the dramatic decrease of Na^+ and Cl^- that occurred during the course of stimulation, Na^+ was still significantly higher in SCC III than in SCC I quarters at t=120 s.

Discussion

Intramammary injection of 100 µg LPS resulted in a considerable inflammatory reaction in the respective quarter, as reported earlier (Bruckmaier et al. 1993; Moussaoui et al. 2002; Schmitz et al. 2002; Yagi et al. 2002). The observed peak of SCC at 12 h after administration of LPS demonstrates that the inflammatory and immunological reaction required a latency period of several hours. Most of the injected LPS would be expected to be removed by the milking at 12 h after application of LPS, since the transient reaction of EC, Na^+ and Cl^- returned to basal levels. Surprisingly, SCC remained elevated until 60 h after LPS application, although tight junction integrity seemed to be largely restored as indicated by Na^+ and Cl^- patterns. It may be speculated that only during periods of high somatic cell diapedesis rates do the tight junctions get leaky enough (Stelwagen et al. 1997; Nguyen & Neville, 1998) to allow considerable movement of ions from blood into milk. This may explain the limited sensitivity of Na^+, Cl^- and EC as indicators of mastitis in the subclinical range (as shown in Expt 2).

The constant SCC in C quarters documents that the elevated SCC in T quarters was a local reaction in response to LPS administration. As expected, EC, Na^+ and Cl^- values in C quarters were numerically elevated at 3 h after application of NaCl solution. For the same reason, the results for T quarters seem to be shifted. However, the dramatic increase of SCC from 0 to 3 h documents the considerable amplitude of the SCC rise as compared with the changes in EC, Na^+ and Cl^- during the impairment of tight junction integrity.

In Expt 2, single quarters with different SCC levels, but without clinical mastitis were used for repeated milk sampling during a teat stimulation of 120 s. For SCC, EC, Na^+ and Cl^-, the values decreased during the sampling period but none of these variables changed before the sample taken at 60 s. It is therefore obvious that the continuous teat stimulation during sampling induced alveolar milk ejection. The onset of milk ejection, i.e., the occurrence of alveolar milk in the mammary cistern, caused a mixture of alveolar and cisternal milk and hence an immediate change of milk ion concentrations (Ontsouka et al. 2003). The lag time between start of stimulation and the start of milk ejection was previously found to be 50–100 s, as a function of the degree of udder filling (Bruckmaier & Hilger, 2001). As a consequence of milk ejection the measurements of EC and of Na^+ and Cl^- concentrations lose sensitivity as mastitis indicators. This fact needs to be considered in the case of foremilk sampling for EC, Na^+ and Cl^- measurements. Foremilk can only be expected to be cisternal milk until about 40 s from the first touch of the udder.

With respect to the differences between SCC III and SCC I quarters, SCC was the most sensitive indicator. The dramatic increase during inflammation was the result of a local immune reaction and mediator-linked active transport of cells into the milk of the respective quarter. In contrast, the rise of Na^+ and Cl^-, and consequently of EC, was a result of diffusion through leaky tight junctions during somatic cell influx. Thus the increment of EC, Na^+ and Cl^- during intramammary inflammation was only a linear shift as compared with the exponential increase of SCC. Therefore, the mixture of cisternal milk with alveolar milk resulted in the disappearance of highly significant differences between SCC I and SCC III quarters for all physicochemical indicators investigated.

It has to be pointed out that the sensitivity of EC, Na^+ and Cl^- was not great enough to distinguish between SCC I and SCC II quarters. In automatic milking systems, the sensitivity of the currently used measurement of EC could be slightly improved by inter-quarter ratio and comparison from one milking to the next even though the EC is measured after the milk ejection.

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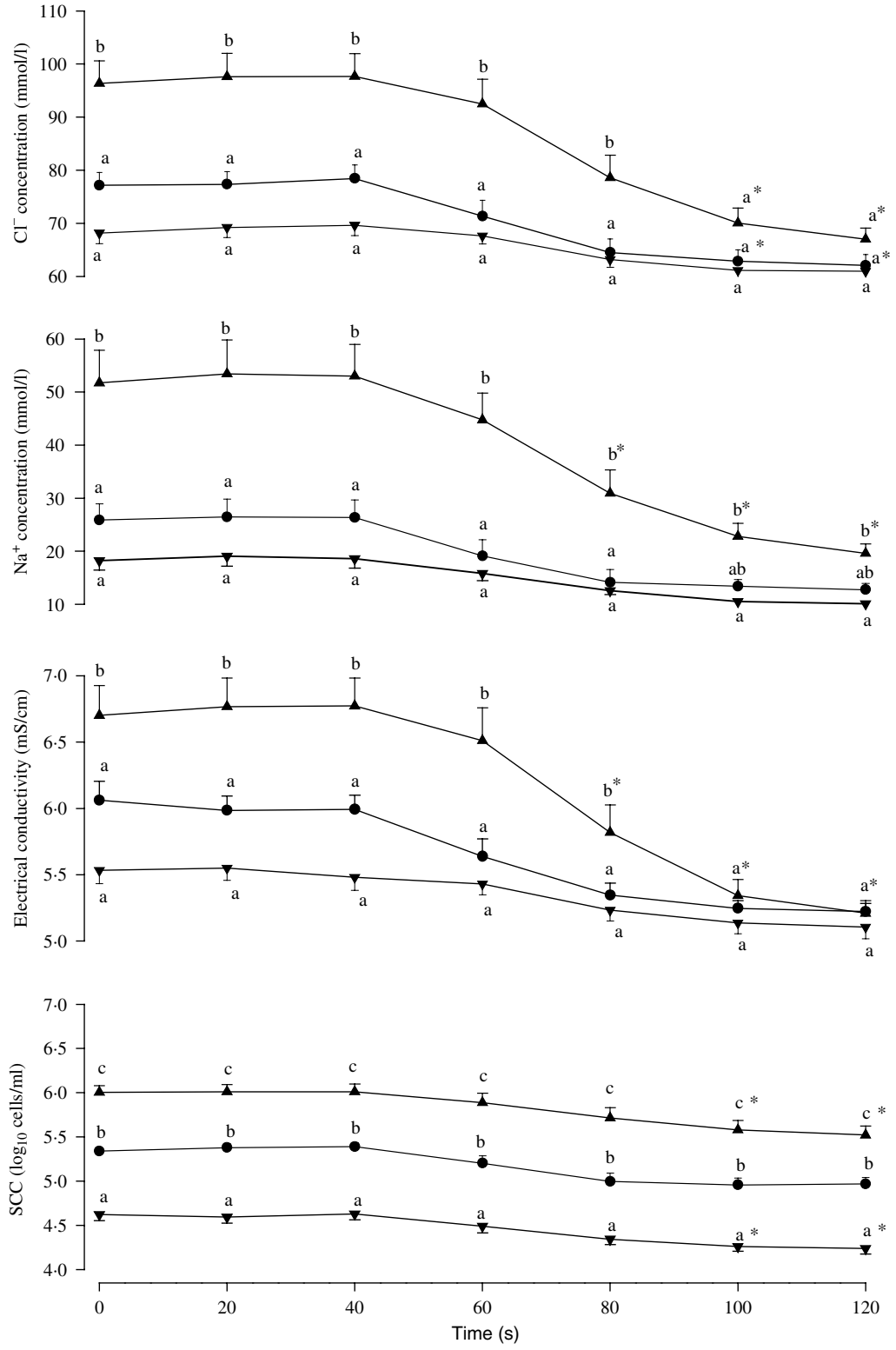

Fig. 2. SCC, EC, Na^+ and Cl^- concentrations in the different milk fractions, 0 to 120 s after the first contact with the udder; data are clustered according to the SCC of the first sample (t = 0 s), ▼ <5·0 log cells/ml (SCC I), ● 5·0–5·7 log cells/ml (SCC II) and ▲ >5·7 log cells/ml (SCC III); n = 20 cows; three milkings each cow; $^{a, b, c}$ means with different letters indicate differences between clusters (P<0·05); * indicates a significant (P<0·05) difference compared with the sample t = 0 s.

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In accordance with the results of Expt 1, only a high somatic cell diapedesis rate during acute inflammation seems to allow electrolyte diffusion from blood into milk, while during the influx of cells at a lower rate the electrolyte concentrations return to their basal levels. Recommended SCC thresholds to distinguish between disturbed and undisturbed milk composition range between 5·0 log cells/ml (Hillerton, 1999) and 5·3 log cells/ml (Smith, 1995). Therefore the studied variables were not satisfactory for distinguishing these thresholds by using the current in-line sampling routine. However, physicochemical indicators could detect serious immune reactions during mastitis.

In conclusion, SCC is the most sensitive measurement to detect mastitis in bovine milk, both before and after the occurrence of milk ejection. In contrast, EC, Na^+ or Cl^- concentrations can be useful tools for in-line measuring but only if quarter milk samples of the cisternal fraction are available. To achieve this goal, foremilk samples must be taken before the occurrence of alveolar milk ejection.

We thank the farms Veitshof and Hirschau, Technical University Munich, for providing the animals and the Milchprüfring Bayern e.V. for analyses of the milk samples. The help of Mr A Knon, Mrs and Mr Ochsendorf during the animal experiments is gratefully acknowledged. The study was partially supported by Bavarian Ministry of Agriculture and Forestry and by a grant from the Hanns-Seidel-Foundation for M Wiedemann.

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