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LEVELS OF GLUCOSE, SERUM ALBUMIN AND SOMATIC CELLS BEFORE AND DURING EARLY STAGES OF ACUTE CLINICAL MASTITIS ARTIFICIALLY INDUCED IN COWS BY MEANS OF HUMAN STRAINS OF GROUP-B STREPTOCOCCI (GBS) ADMINISTERED INTRACISTERNALLY

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Abstract

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The investigation was performed on 3 cows, sampled repeatedly before and during the initial 48 h of artificially induced, acute, clinical mastitis. The results of the investigation both augment and support those of earlier work on the levels and significant correlations of glucose, serum albumin and somatic cells in normal and abnormal secretions monitored before and after the usual milking of healthy lactating cows had been suspended.

During acute mastitis, udder secretions from artificially infected quarters showed highly significant escalations of somatic cell counts which coincided with equally significant increases on a high and intermediate level of serum albumin values in both the infected and non-infected quarters. Corresponding glucose values fluctuated from 0,07–0,22 and 0,18–0,32 mM in the former and latter quarters respectively.

The selective and elevated transfer of serum albumin in otherwise unaffected quarters of acutely mastitic udders suggests rather specific collateral vascular and epithelial changes of unknown nature and magnitude.

The data indicate that marked fluctuations of glucose may occur within and between quarters of individual and different cows respectively. Such variations could significantly affect phagocytosis and killing of bacteria challenging the intramammary leucocytic udder barrier before and particularly during manifestation of mastitis. Hence, udder health, although dependent on specific natural defence mechanisms such as the leucocytes and related systems in milk, may depend even more significantly on the supplies of glucose to and within the bovine mammary gland.

Résumé

TAUX DE GLUCOSE, SERUM ALBUMINE ET CELLULES SOMATIQUES AVANT ET PENDANT LES STADES DE DÉBUT DE MAMMITE CLINIQUE AIGUË CHEZ LA VACHE ARTIFICIELLEMENT PROVOQUÉE, AU MOYEN DE SOUCHES HUMAINES DE STREPTOCOQUES DE GROUPE-B (GBS) ADMINISTRÉS INTRACISTERNELLEMENT

L'investigation fut pratiquée sur trois vaches échantillonnées de manière répétée avant et pendant les 48 heures initiales de la provocation artificielle d'une mammite clinique aiguë. Les résultats de l'investigation augmentent et soutiennent ceux obtenus par des travaux antérieurs sur les taux et les corrélations significatives de glucose, de serum albumine et des cellules somatiques dans les sécrétions normales et anormales controlée avant et après que la traite habituelle des vaches saines en lactation avait été suspendue.

Pendant une mammite aiguë les sécrétions du pis des quartiers artificiellement infectés montrèrent des élévations hautement significatives des comptes de cellules somatiques qui coincidèrent avec des augmentations également significatives sur un niveau élevé et intermédiaire des taux de serum albumine tant dans les quartiers infectés que dans ceux non-infectés. Les valeurs glucose correspondantes fluctuèrent de 0,07–0,22 et 0,18–0,32 mM dans les premiers et derniers quartiers respectivement.

Le transfert sélectif et élevé du serum albumine dans les quartiers d'autrepart inaffectés du pis à mammite aiguë, suggère plutôt des changements épithéliaires et vasculaires collatéraux spécifiques de nature et de magnitude inconnues.

Les données indiquent que des fluctuations marquées de glucose peuvent survenir dans et entre les quartiers de vaches individuelles et différentes respectivement. De telles variations pourraient affecter de manière significative la phagocytose et la destruction des bactéries qui s'opposent à la barrière leucocytaire intramammaire avant et particulièrement pendant la manifestation de la mammite. De là, la santé du pis, bien que dépendante de mécanismes de défense naturels spécifiques, tels que les leucocytes et systèmes apparentés dans le lait, peut même dépendre plus significativement des apports de glucose à, et dans la mamelle bovine.

Introduction

Recent literature reflects a renewed assessment of the role of several factors in the natural defence mechanisms against bacterial infection of the bovine mammary gland. One of the major systems of defence under investigation is the leucocytic udder barrier (Schalm, Carroll & Jain, 1971; Paape & Wergin, 1977; Paape, Wergin, Guidry & Pearson, 1979). Although there seems to be a general consensus on the practical significance of the barrier (Giesecke & Van den Heever, 1974; Newbould, 1974; Paape, Schultze & Kortum, 1979), *in vivo* (Schalm *et al.*, 1971) and *in vitro* investigations (Wisniowski, Romaniukowa & Grajewski, 1965; Kent & Newbould, 1969; Russell & Reiter, 1975) point to considerable deficiencies presumably affecting the intramammary leucocytic defence system *in vivo*.

Available data indicate that the defensive efficacy of the leucocytic udder barrier may depend on qualitative and/or quantitative characteristics of factors related to (1) the intramammary environment surrounding leucocytes in milk and (2) others inherent in the polymorphonuclear neutrophilic (PMN) leucocytes.

The former may include opsonins and related humoral factors (Wisniowski *et al.*, 1965; Newbould, 1973; Russell & Reiter, 1975; Guidry, Paape & Pearson, 1977a, b), phagocytizable casein micelles and fat globules (Russell & Reiter, 1975; Russell, Brooker & Reiter, 1976, 1977; Paape, Guidry, Kirk &

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Bolt, 1975; Paape & Guidry, 1977; Paape & Wergin, 1977; Wergin & Paape, 1977) and certain hormones (Paape, Kral, O'Brien & Schultze, 1971; Aström, 1972; Guidry, Paape & Pearson, 1975, 1976; Gwazdauskas, Paape & McGilliard, 1977; Paape, Desjardins, Guidry, Miller & Smith, 1977), whereas the latter may involve factors such as concentration per mℓ of milk of competently phagocytizing PMN-leucocytes (Schalm *et al.*, 1971), loss or atrophy of certain organelles (Anderson, 1979), limited regeneration of plasma membrane (Paape *et al.*, 1979), limited requirement of oxygen for synthesis of H_2O_2 (Iyer, Islam & Questel, 1961; Klebanoff, 1968; Paul & Sbarra, 1968; Erslev & Gabuzda, 1974; Schalm, Jain & Carroll, 1975; Anderson, 1979; Paape *et al.*, 1979), reduced intracellular glycogen reserves (Naidu & Newbould, 1973), adjusting of pH-values (Reinitz & Paape, 1979) and general viability of PMN-leucocytes (Newbould, 1974), major requirement of glucose for synthesizing ATP, H_2O_2 and lactic acid (Lehninger, 1970; Diem & Lentner, 1971; Bainton, Nichols & Farquahar, 1976).

It seems of particular interest that glucose should be freely available in bovine milk at concentrations of 13,8–18,56 mg per 100 mℓ (Reineccius, Kavanagh & Keeney, 1970; Mackie, Giesecke, Lück & De Villiers, 1977). Diem & Lentner (1971) suggest that 10^{11} human leucocytes normally metabolize 4,0 mM of oxygen and 14 mM of glucose per hour, whereas some 30,1 mM of lactate are synthesized during the same time. From their investigations on normally lactating and artificially involuting healthy bovine udders, Mackie *et al.* (1977) concluded that regression coincided with highly significant correlations between PMN-leucocytes and lactate (r=0,78) and PMN-leucocytes and glucose (r=—0,89) and respectively a twenty- to forty-fold decrease of intramammary oxygen supplies. In terms of research on bovine mastitis, Newbould (1970a, b; 1973) showed that PMN-leucocytes from milk responded *in vitro* with elevated phagocytosis after glucose was added to the substrate. Bogin, Ziv, Avidar, Rivetz, Gordin & Saran (1977) determined significant correlations between escalating levels of cellular deterioration, PMN-leucocytes and lactate on udder secretions from different types of mastitis. Jain & Lasmanis (1978) suggested that reduced phagocytic activity of PMN-leucocytes in milk may depend on limited lacteal levels of glucose, while Müller & Berchtold (1980) more recently reported on highly significant improvements of coliform mastitis treated with a combination of antibiotic and glucose.

All the above suggest that the efficacy of the leucocytic udder barrier apparently would depend not only on the individual factors already mentioned but also on the level in milk of glucose available for supplementing the leucocytic functions.

For a better understanding of the general significance of glucose levels in bovine udder secretions and to augment the earlier work by Mackie *et al.*, (1977) and other workers, glucose levels were determined in normal and mastitic udder secretions.

Materials and methods

The different determinations were performed as part of an investigation on the udder-pathogenicity of human strains of Group-B streptococci, and details of the methods have been described elsewhere (Van den Heever & Giesecke, 1980). Where further detail seemed essential, such information is provided below.

Experimental animals

Three grade Friesland cows, differing in age, number of lactations, daily milk yield and stage of lactation, were used. They were clinically healthy, in good condition and free from tuberculosis and brucellosis. The cows were milked daily by machine at 08h00 and 14h00.

Routine and method of sampling

Before sampling, udders were clinically examined by inspection and palpation and the mammary secretions were evaluated by means of the strip-cup method.

Foremilk samples required for the different determinations were collected aseptically from the individual quarters on days —21, —14, —7 and 0 before and at 6, 24, 30 and 48 h after septic mastitis had been artificially induced.

After milk samples had been collected by a commonly accepted method (Giesecke & Viljoen, 1974), a sterile swab sample was taken from each sample, and an 0,1 mℓ aliquot from each milk sample was transferred for deproteinizing to test-tubes containing 1,0 mℓ of uranyl acetate*. The remainder of the samples were kept for cytological and immunochemical determinations.

Laboratory examination of milk

The swab samples were used for preparing bacteriological cultures by methods already described (Giesecke, Nel & Van den Heever, 1968).

The deproteinized samples were centrifuged at 3500 g for 15 min at 5 °C. An aliquot of 0,2 mℓ of the clear supernatant fluid was then removed from beneath the fat layer of each sample for determining the glucose content by the GOD-Perid* method. A spectrophotometer** with a slit width of 1 nm, a wavelength of 610 nm and 10 mm glass cuvettes was used.

The electronic cell count (ECC) and levels of bovine serum albumin (BSA) were determined as described by Giesecke & Viljoen (1974).

Statistical analyses

The results were subjected to a one-way analysis of variance and determinations of Bonferroni's Smallest Significant Difference for assessing the level of significance of differences between the 2 corresponding mean values compared. For convenience, such values were identified by means of serial numbers 1–9 in Table 1 with its corresponding matrix of significant differences.

Results

The inoculation of quarters with human strains of GBS resulted in acute clinical mastitis in all inoculated quarters as described elsewhere (Van den Heever & Giesecke, 1980). The clinical symptoms coincided with fluctuating values of glucose, BSA and ECC, especially, but not exclusively, in the udder secretions of the inoculated quarters (Fig. 1–3).

From the illustrations it is apparent that, depending on the individual cow, appreciable changes in glucose, BSA and ECC levels occurred in both the inoculated and non-inoculated quarters (Fig. 4; Table 1).

* Boehringer, Mannheim
** Varian model Super Scan 2

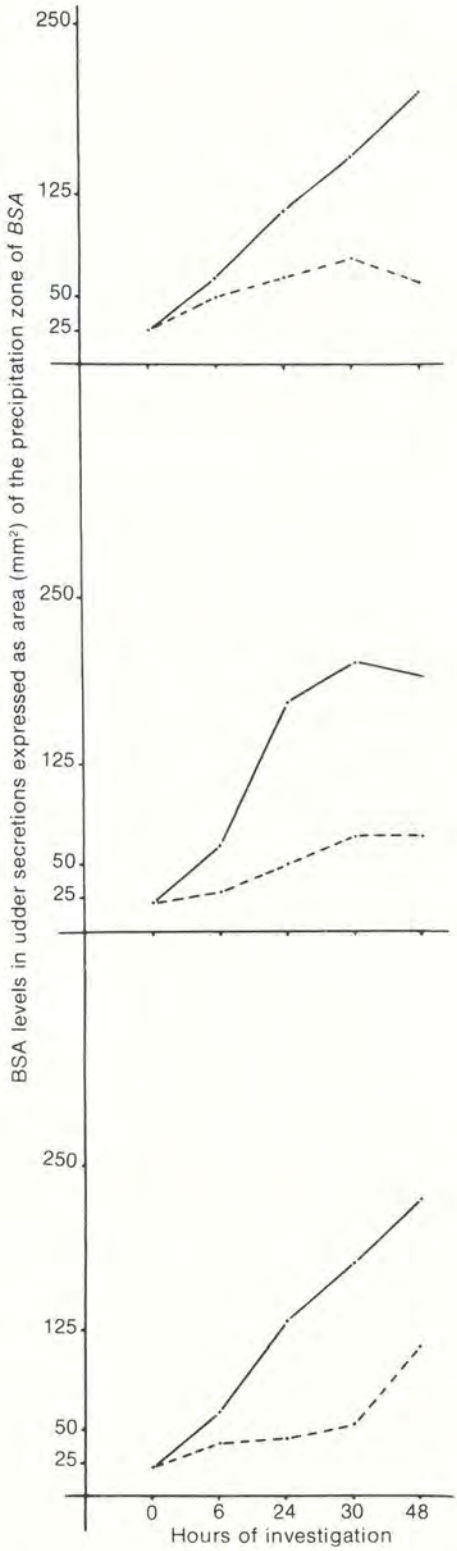

FIG. 1 Changes in infected (·———·) and non-infected (·– – –·) quarters of individual cows (No. 655, 8718, 918 from top graph to bottom) of BSA-levels expressed as area (mm²) of their precipitation zones (N.B. An area of 50,27 mm²=critical level of BSA proposed by Giesecke & Viljoen (1974) for distinguishing normal and mastitic udder secretions)

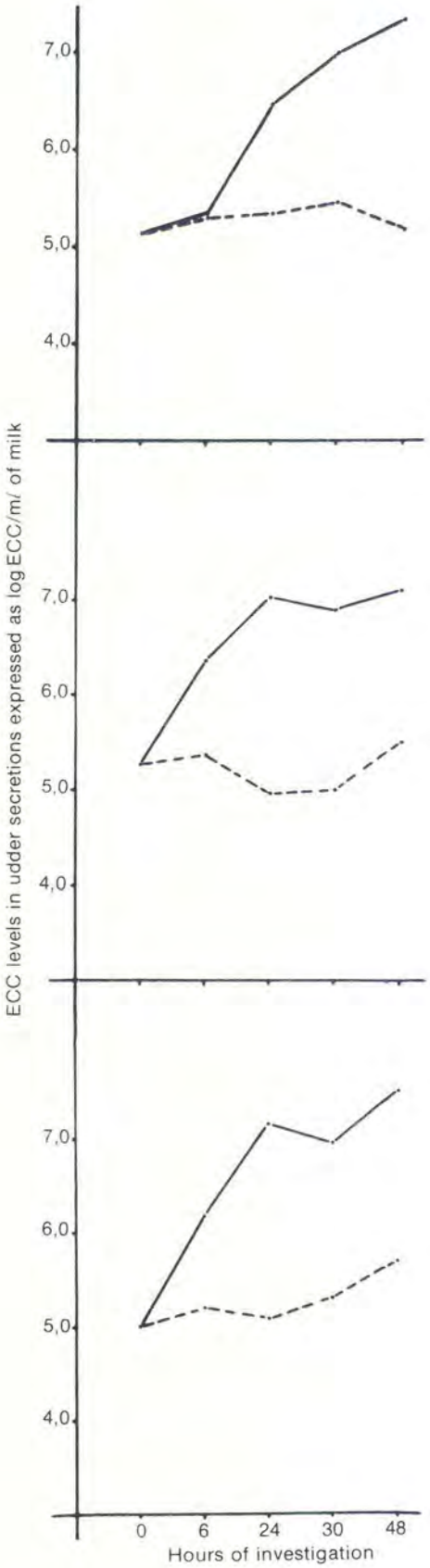

FIG. 2 Changes in infected (·———·) and non-infected (·– – –·) quarters of individual cows (No. 655, 8718, 918 from top graph to bottom) of ECC-levels expressed as log ECC/mℓ of udder secretion (N.B. A log ECC/mℓ of milk of 5,7=critical level of ECC usually used for distinguishing normal and mastitic udder secretions)

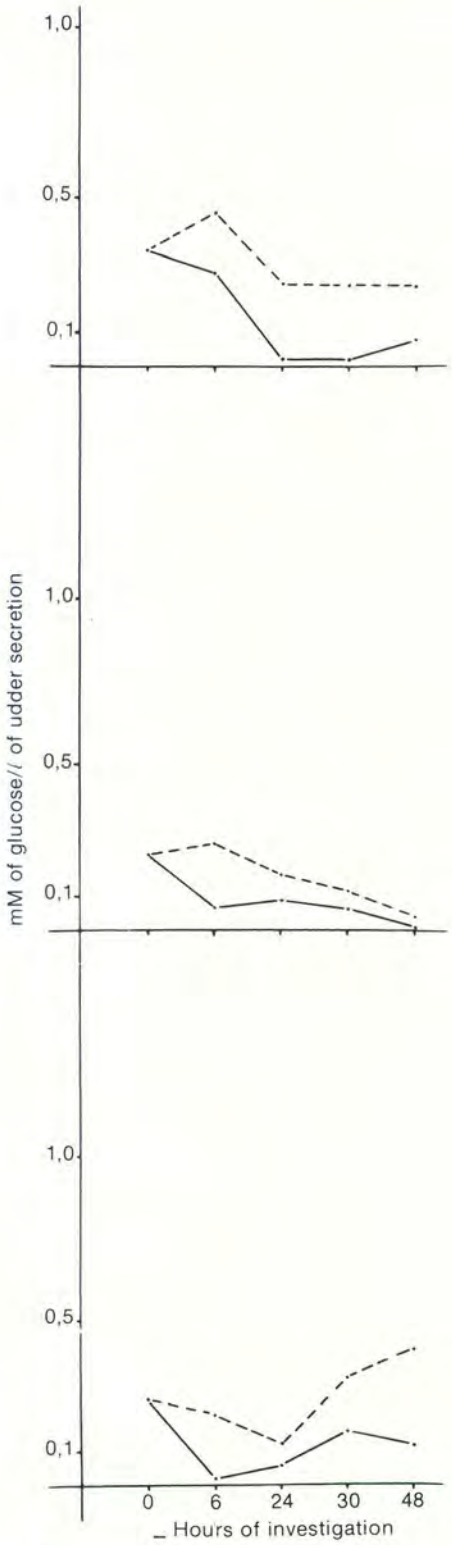

FIG. 3 Changes in infected (•———•) and non-infected (•– – –•) quarters of individual cows (No. 655, 8718, 918 from top graph to bottom) of glucose levels expressed as mM/ℓ of udder secretion

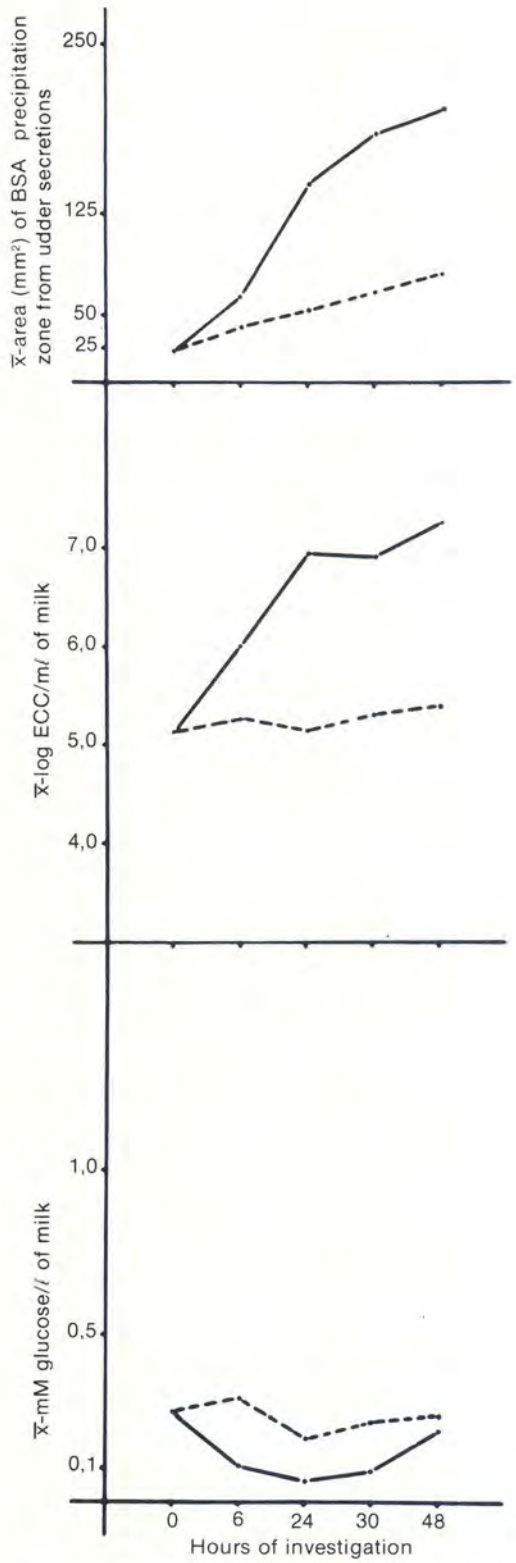

FIG. 4 Mean changes ($\bar{x}$) observed on the levels of BSA, ECC and glucose in the udder secretions of infected (•———•) and non-infected (•– – –•) quarters of 3 cows

TABLE 1 Changes observed before and during artificial acute clinical mastitis on lacteal mean-values of BSA, ECC and glucose and the statistical significance of the fluctuations of the BSA and ECC values

State of quarters	Designations	Periods of investigation/$\bar{x}$-values observed				
		Days −21; −14; −7 and 0 collective	Days (hours)			
			0,25 (6)	1,0 (24)	1,25 (30)	2,0 (48)
Non-infected.....	n-quarters.....	45	8	8	8	8
	BSA (mm^2).....	22,82	41,45	52,67	64,72	83,18
	ECC (log).....	5,142	5,268	5,158	5,315	5,478
	Glucose (mM).....	0,27	0,32	0,18	0,24	0,26
	serial number of value.....	1	3	5	7	9
Infected.....	n-quarters.....	—	4	4	4	4
	BSA (mm^2).....	—	63,62	146,48	183,59	202,24
	ECC (log).....	—	6,068	6,928	6,928	7,263
	Glucose (mM).....	—	0,11	0,07	0,09	0,22
	serial number of value.....	—	2	4	6	8

Matrix on levels of significance (*P<0,05; **P<0,01) of Bonferroni's Smallest Significant Differences between corresponding values identified by means of their respective serial numbers

Serial number of values	BSA values								ECC values							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
2.....	**								**							
3.....										**						
4.....	**	**	**						**	*	**					
5.....	**			**						**		**				
6.....	**	**	**		**				**	*	**		**			
7.....	**		*	**		**				*		**		**		
8.....	**	**	**	**	**		**		**	**	**		**		**	
9.....	**		**	**	*	**		**				**		**		**

The mean values obtained from corresponding data from the 3 cows suggested a tendency of glucose levels temporarily to decrease in the secretions from the affected quarters. Comparisons of these changes with those of the non-inoculated control quarters did not indicate statistically significant differences, presumably because of the large variations between the cows. In contrast, however, highly significant changes of BSA-levels occurred both within and between the inoculated and non-inoculated quarters, whereas comparably significant fluctuations of the ECC values were found only in the inoculated quarters.

Discussion

Previous investigations on the diagnosis of mastitis (Giesecke, 1974; Giesecke & Viljoen, 1974) suggested that the combined use of BSA and ECC as criteria for epithelial damages and inflammatory cell reactions had considerable advantages. Subsequent investigations (Smith, Chesworth, Henderson & Rodway, 1979; Gudding, 1980; Senft, Meyer & Erhardt, 1980) and present data support such a contention, especially as it is also apparent from other work (Giesecke & Van den Heever, 1974; Thieme & Haasmann, 1978) that elevated somatic cell counts may occur in milk from cows and udders affected by conditions other than mastitis. On the other hand, further investigations on BSA-levels in milk by Giesecke & Van den Heever (1975) revealed that abnormal BSA values could occur despite normal ECC values. Such findings, though rare, seem contradictory and cannot yet be readily explained. It is thus noteworthy that during the 48 h of the present investigation, the non-infected quarters showed significantly raised BSA values in the presence of insignificant fluctuations within the normal range of the corresponding ECC values and glucose levels. Data on premature mammary regression (Mackie *et al.*, 1977) suggest that such changes are probably related, not to regression, as was thought earlier (Giesecke & Van den Heever, 1975), but to collateral vascular and epithelial changes favouring a selectively elevated transfer of BSA in otherwise apparently unaffected quarters of an acutely mastitic udder.

Earlier investigations relating to glucose (Reineccius *et al.*, 1970; Mackie *et al.*, 1977) and present data clearly suggest that udder secretions from normal, regressive or mastitic bovine udders contain fluctuating levels of free glucose. Significant correlations found between PMN-leucocytes, lactate and glucose (Mackie *et al.*, 1977) further indicate that, at least during regression, major quantities of glucose in milk are metabolized to lactate by the PMN-leucocytes present.

Biochemical (Bogin *et al.*, 1977) and electron microscopic investigations on normal and mastitic bovine and murine mammary glands (Chandler, 1970a, b; Chandler, Reid, Harrison & France, 1974; Hollmann, 1974; Anderson & Chandler, 1975; Reid, Harrison & Anderson, 1976) further suggest that septic mastitis, especially during the acute stages, coincides but is by no means synonymous with intramammary epithelial regression. Hence it seems reasonable to conclude that PMN-leucocytes present in mastitic udder secretions most probably metabolize glucose to lactate as rapidly as cells in secretions from regressive udders.

From the value of 14,0 mM of glucose normally required per hour by 10^{11} human leucocytes (Diem & Lentner, 1971), from fluctuating glucose levels of $1,03 \pm 0,7$ mM observed in bovine milk by Mackie *et al.* (1977) and especially from the low glucose concentration of 0,27 mM determined during the present investigation, it seems conceivable, however, that under certain conditions a level of glucose, almost negligibly low relative to its supply and demand, may occur, even in an otherwise normal udder. In such circumstances, glucose deficiency in milk may aggravate the aforementioned deficiencies inherent in PMN-leucocytes in milk. Level and turnover of glucose in milk could thus be of major significance to the leucocytic defence of the bovine mammary gland.

Periodic intramammary shortages of glucose may account for the generally accepted elevated susceptibility to mastitis known to occur under conditions associated with: (a) beginning and end of lactation; (b) irregular and incomplete milking; (c) certain climatic, weather, seasonal and feeding conditions; (d) febrile diseases other than acute mastitis and other comparable situations related to stress and poor management. The data therefore indicate that udder health, although dependent on more specific natural defence mechanisms, such as the PMN-leucocytes and related systems in milk, may depend even more significantly on the supplies of glucose to and within the bovine mammary gland. Intramammary deficiencies of glucose presumably would affect the PMN-leucocytes on different levels such as the leucocytic energy metabolism, synthesis of H_2O_2 and lactic acid, lowering of the pH in the cell and its phagosomes and related intracellular activities. Such aberrations would significantly affect efficient phagocytosis and the killing of bacteria challenging the intramammary leucocytic system before and particularly during the manifestation of mastitis.

The very low values of 0,07–0,09 mM of glucose observed during the present investigation in the secretions of the infected and acutely mastitic quarters are indications of glucose depletion. Alleviation during intramammary mastitis therapy of comparable depletions of glucose seems to explain the more successful treatments by means of glucose/antibiotic combinations of coliform mastitis reported by Müller & Berchtold (1980).

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