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UDDER HEALTH IMPLICATIONS OF PREMATURE BOVINE MAMMARY REGRESSION. II. COLLATERAL INTRAMAMMARY REACTIONS IN QUARTERS MILKED NORMALLY DURING 168-HOUR MILK STATIS IN OPPOSITE QUARTERS

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ABSTRACT

GIESECKE, W. H., KORYBUT-WORONIECKI, P. A. & KOWALSKI, Z. E., 1990. Udder health implications of premature bovine mammary regression. II. Collateral intramammary reactions in quarters milked normally during 168-hour milk stastis in opposite quarters. *Onderstepoort Journal of Veterinary Research* 57, 49–56 (1990)

This investigation has shown that collateral intramammary reactions (CIR's) occur when secretory disturbances in one quarter lead to corresponding changes in one or more of the other, unperturbed, quarters of the same udder. Compared with normal baseline values, the mean values and variations of bovine serum albumin (BSA), *beta*-N-acetyl-D-glucosaminidase (NAG), mannose (MAN), galactose (GAL) and glucose (GLU) showed slight increases under CIR conditions. Fluctuations of the carbohydrates preceded changes of somatic cell counts (SCC), BSA and NAG in milk. SCC and NAG showed significant negative correlations with MAN, GAL and GLU, the latter being the only parameter significantly correlated with each of the other parameters.

The data suggest that the cow's stress control system responded to the milk stasis in the unmilked quarters, by initiating a form of general stress that affected the whole udder. This aggravated regressive conditions in the unmilked quarters and provoked CIR's in the normally-milked quarters. The CIR's amounted to a transient, regressive, compensatory re-adjustment of lactational homeostasis under conditions of acute general stress.

CIR's similar to those investigated could also occur in close association with other local and general stressful conditions, disturb the natural defence of the udder and account for fluctuating mammary resistance to bacterial infection.

Key words: Collateral intramammary reactions, stress, susceptibility to intramammary infection, udder health in dairy cattle.

INTRODUCTION

In an earlier report, Giesecke (1975) indicated that lactating cows, with infectious subclinical mastitis in one or more quarters, showed SCC's in milk from normal quarters which were increased within normal ranges by 43 % above mean baseline values. Subsequent research on acute clinical mastitis (Giesecke & Van den Heever, 1981) supported the possibility of collateral SCC reactions. It was shown that acute mastitis coincided with significant fluctuations of lacteal levels of GLU and BSA in milk from the uninfected, apparently normal quarters.

Such initial data suggested that CIR's may occur when stressful conditions in 1 or more of the 4 quarters stimulate corresponding, though usually less distinct, changes in the other, apparently unaffected, normal quarter(s). No other reference to CIR's in dairy cows appears in current literature.

Research on various aspects of stress in udder health (Giesecke, 1985a; Giesecke, Van Staden, Barnard & Petzer, 1988), mammary regression (Mackie, Giesecke, Lück & De Villiers, 1977; Giesecke, 1985a, b), dynamic fluctuations of subclinical udder conditions (Giesecke & Barnard, 1985; Giesecke, 1988) and on the pathogenesis of subclinical mastitis (Giesecke & Barnard, 1986) suggests that low-level fluctuations of several lacteal parameters occur frequently, are closely comparable with CIR's and might predispose udders to bacterial invasion and infection.

Futhermore, under field conditions cows with clinical mastitits in 1 udder quarter tend to be susceptible, even under comparatively satisfactory hygienic conditions, to the "jumping" of mastitis to other apparently normal quarters. These frequently start to show elevated SCC's and other signs of subclinical udder irritation or mastitis. Such CIR's often involve increased lacteal levels of BSA, indicating elevated epithelial permeability to serum proteins and lacteal abnormalities which are known to promote, under *in vitro* conditions, the growth in milk and whey of pathogenic bacteria (Brown, Baetz & McDonald, 1983; Mattila, Maisi & Sandholm, 1984; Mattila, Syväjärvi & Sandholm, 1984; Mattila & Sandholm, 1985; Mattila, Kaartinen & Sandholm, 1986; Kaartinen & Sandholm, 1986).

From the above discussion it is apparent that the practical implications of CIR's cannot be assessed without further data on their triggering, development and related characteristics. The aim of the present investigation was, to determine under CIR conditions the characteristics of lacteal levels of SCC, BSA, NAG, LAC, MAN, GAL and GLU, as well as their implications for the initial triggering and further development of the CIR's.

MATERIALS AND METHODS

This investigation involved the same cows (apart from 1 cow with intramammary infection), and was conducted under the conditions of research, statistical analyses and terminology already described in the preceding paper by Giesecke, Korybut-Woroniecki & Kowalski (1990). The CIR's were monitored in the left (L) udder quarters milked normally as controls when, at the same time, premature regression was induced in the opposing right (R) quarters by the suspension of milking. Consequently, the CIR's were determined in the left quarters after 24, 48, 72, 120 and 168 h of milk statis in the corresponding right udder halves (Table 1).

The lacteal levels of SCC, BSA, NAG, LAC, MAN, GAL and GLU monitored during Periods I and II (Table 1), have been evaluated below from the point of view of aspects of the mammary regression elucidated by Giesecke *et al.* (1989).

RESULTS

Udder health during Period I

During Period I, milk from the left udder quarters showed normal values of SCC, BSA, NAG, LAC, MAN, GAL and GLU (Table 2). The lacteal concentrations of all parameters except LAC showed marked variations. Correlations differed in their

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TABLE 1 Schedule of days, samplings and hours of investigation

Day	Period	Sampling	Hours	Purpose of sample
−6	I	1	−144	Separate pooling of values from all untreated right and left udder quarters to determine mean baseline values applicable to each udder half
−4	I	2	−96	
−2	I	3	−48	
−1	I	4	−24	
0	I	5	0	
1	II	6	24	Separate pooling of values from treated right and normally milked left quarters to assess mean changes in opposite udder halves under different conditions of treatment
2	II	7	48	
3	II	8	72	
5	II	9	120	
7	II	10	168	

TABLE 2 Pooled mean ($\bar{x}$) values, standard deviations (SD), coefficients of variation (CV %) and correlation matrix of parameters monitored in 40 milk samples collected during Period I from the left udder quarters

Parameters	Statistical designation values			Parameters × coefficients of correlation (r)*						
	$\bar{x}$	SD	CV %	SCC	BSA	NAG	LAC	MAN	GAL	GLU
SCC per mℓ	157 200	131 854	83,9	1,00						
BSA mg per mℓ	0,1769	0,0920	52,0	−0,21	1,00					
NAG units of activity	23,9950	18,2081	75,9	0,12	0,04	1,00				
LAC µmol per mℓ	192,9438	11,4207	5,9	0,07	−0,42	−0,17	1,00			
MAN µmol per mℓ	0,0219	0,0100	45,7	−0,18	−0,02	−0,36	0,39	1,00		
GAL µmol per mℓ	0,2640	0,0940	35,6	−0,27	−0,12	−0,46	0,40	0,91	1,00	
GLU µmol per mℓ	0,1014	0,0553	54,5	−0,12	−0,27	−0,38	0,51	0,75	0,65	1,00

* Limits of correlation coefficients at different levels of significance: r = 0,2573 (P<0,1), r = 0,3044 (P<0,05), r = 0,3578 (P<0,02), r = 0,3932(P<0,01), r = 0,4896 (P<0,001)

TABLE 3 Mean values of parameters monitored in milk from 8 left udder quarters milked normally during 168 h of milk stasis in the opposite right udder halves

Parameters	Mean baseline values of Period I	Hours of milk stasis in the right udder half × corresponding mean values monitored in milk from left udder half				
		24	48	72	120	168
SCC per mℓ	157 200	149 500	203 750	215 625	60 750	128 250
BSA mg per mℓ	0,1769	0,174	0,202	0,253	0,180	0,160
NAG units of activity	23,9950	28,288	35,175	34,975	46,675	18,350
LAC µmol per mℓ	192,9438	259,130	156,078	156,865	150,557	116,242
MAN µmol per mℓ	0,0219	0,038	0,021	0,019	0,022	0,022
GAL µmol per mℓ	0,2640	0,535	0,253	0,252	0,262	0,252
GLU µmol per mℓ	0,1014	0,171	0,067	0,095	0,092	0,120

TABLE 4 Percentage (%) variation from baseline values of parameters monitored in milk from 8 left udder quarters during 168 h of milk stasis in the opposite udder halves

Parameters	Hours × percentage (%) deviations from baseline				
	24	48	72	120	168
SCC per mℓ	−4,9	29,6	37,2	−61,4	−18,4
BSA mg per mℓ	−1,6	14,2	43,0	1,7	−9,6
NAG units of activity	17,9	46,6	45,8	94,5	−23,5
LAC µmol per mℓ	34,3	−19,1	−18,7	−22,0	−39,8
MAN µmol per mℓ	73,5	−4,1	−13,2	0,5	0,5
GAL µmol per mℓ	102,7	−4,2	−4,5	−0,8	−4,5
GLU µmol per mℓ	68,6	−33,9	−6,3	−9,3	18,3

TABLE 5 Percentage (%) variation from mean levels in milk of the preceding day monitored in milk from 8 left udder quarters during 168 h of milk stasis in the opposite right udder halves

Parameters	Hours × percentage (%) changes relative to preceding value				
	24	48	72	120	168
SCC per mℓ	−4,9	36,3	5,8	−71,8	111,1
BSA mg per mℓ	−1,6	16,1	25,3	−25,9	−11,1
NAG units of activity	17,9	24,3	−0,6	33,5	−60,7
LAC µmol per mℓ	34,3	−39,8	0,5	−4,0	−22,8
MAN µmol per mℓ	73,5	−44,7	−9,5	15,8	0,0
GAL µmol per mℓ	102,7	−52,7	−0,4	−4,0	−3,8
GLU µmol per mℓ	68,6	−60,8	41,8	−3,2	30,4

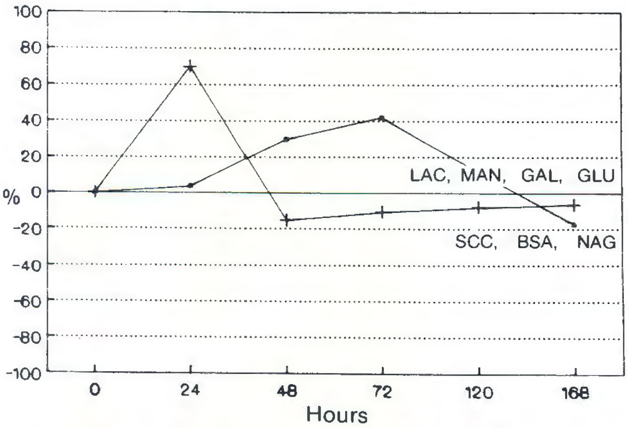

FIG. 1 Major mean patterns of comparative (%) changes from baseline of udder health (+; SCC, BSA, NAG) and metabolic (•; LAC, MAN, GAL, GLU) parameters monitored during 168 h of collateral intramammary reactions in the left udder quarters

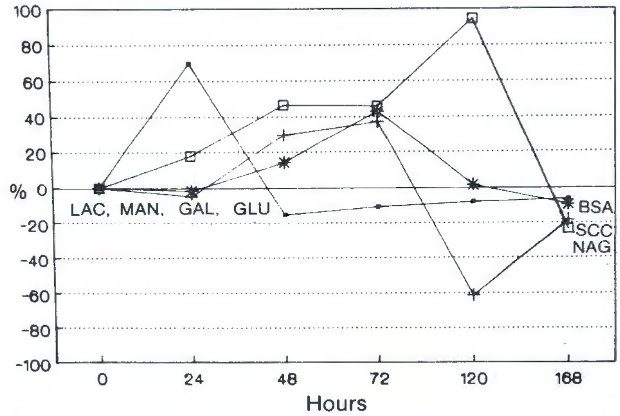

FIG. 2 Percentages (%) changes from baseline of SCC (+), BSA (*) and NAG (□) levels compared individually with the corresponding mean pattern of the combined fluctuations of LAC, MAN, GAL and GLU (•) monitored during 168 h of collateral intramammary reactions in the left udder quarters

levels of significance. It is noteworthy that at the normal baseline level of Period I, the parameters NAG and BSA were more closely correlated with some of the reducing sugars than with the SCC values. The different carbohydrate combinations were correlated highly significantly (Table 2).

Collateral intramammary reactions during Period II

Daily fluctuations of the lacteal concentrations of the parameters revealed more detailed aspects of the CIR's (Table 3–5).

For example, the 24, 48, 72, 120 and 168 h values of the SCC (Table 3) deviated from baseline by −4,9 %, 29,6 %, 37,2 %, −61,4 % and −18,4 % respectively (Table 4). The other parameters also fluctuated, but each of them differently. Such differences became still more apparent from the precentage changes which indicate the comparative degree of daily fluctuations (Table 5).

The initial sharp increases and decreases at 24 and 48 h respectively of LAC, MAN, GAL and GLU (Table 5) indicated distinct early adjustments of the epithelial carbohydrate metabolism of the L udder halves to the stasis perturbation in the opposing R udder halves. The GLU fluctuations showed, for example, an initial increase of 68,6 % and decrease of 60,8 % (Table 5).

LAC levels fluctuated abnormally from baseline (Table 4) during the 24–168 h, whereas the other parameters exceeded normal ranges of variation (Table 2) only from 24–48 h (Table 4).

The fluctuations of the lacteal concentrations of LAC, MAN, GAL and GLU (= metabolic parameters) tended to precede fluctuations of SCC, BSA and NAG (= udder health parameters) (Fig. 1).

The major increases in the carbohydrates at 24 h preceded the increases of SCC, BSA and NAG (Fig. 2), whereas major decreases of carbohydrate levels at 48 h preceded the peaking of SCC and BSA at 72 h, and of NAG at 120 h (Fig. 2).

This broad pattern of events was associated with closer correlations of several parameter combinations (Table 6).

Levels of significance of the correlation coefficients (Table 6) differed depending on parameter combinations. Similar to the correlations of the baseline values (Table 2), the lacteal levels of NAG and BSA during Period II were more closely correlated with certain carbohydrates than with the SCC.

The SCC and several of the carbohydrates were more closely correlated during Period II (Table 6) than during Period I (Table 2).

Transmission of signals of intramammary perturbation during Period II

The concentrations of reducing sugars in udder secretions from the unmilked and milked quarters showed fluctuations which tended to be rather similar in nature but different in magnitude (Fig. 3 & 4).

Parallel patterns of the changing concentrations of GLU (Fig. 3) and LAC (Fig. 4) in milk from the R and L udder halves suggest that signals of intramammary perturbation were transmitted from the unmilked to the milked udder quarters.

TABLE 6 Correlation matrix of parameters monitored in milk from 8 left udder quarters milked normally during 168 h of milk stasis in the opposite right udder halves

Parameters	Parameters × correlation coefficients (r)*						
	SCC	BSA	NAG	LAC	MAN	GAL	GLU
SCC	1,00						
BSA	0,15	1,00					
NAG	0,33	0,19	1,00				
LAC	0,13	0,04	0,13	1,00			
MAN	−0,33	0,02	−0,37	0,44	1,00		
GAL	−0,34	−0,04	−0,35	0,61	0,93	1,00	
GLU	−0,30	−0,40	−0,50	0,30	0,79	0,74	1,00

* For levels of significance see Table 2

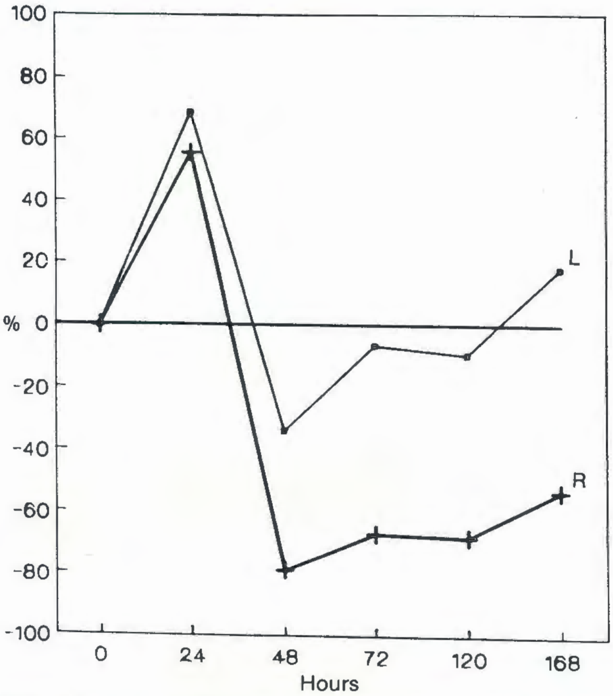

FIG. 3 Percentage (%) glucose variations from baseline in milk from L (•) and R (+) quarters during Period II.

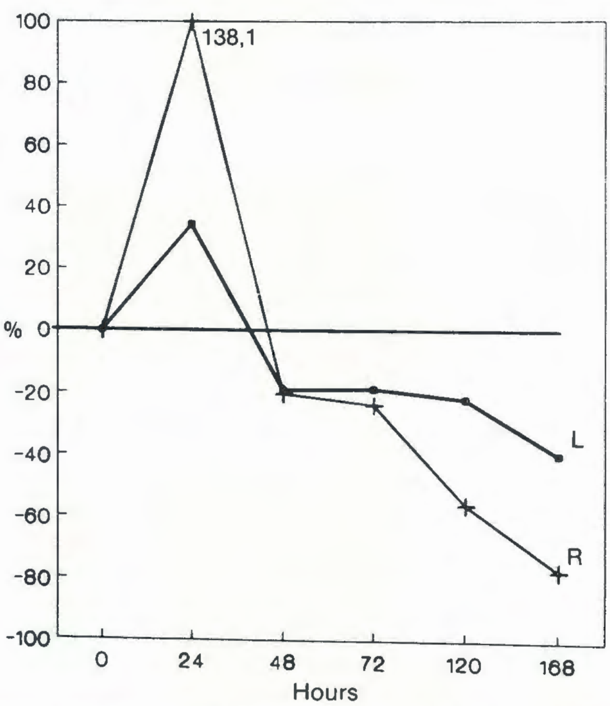

FIG. 4 Percentage (%) lactose variations from baseline in milk from L (•) and R (+) quarters during Period II

DISCUSSIONS

What few data are available on the CIR's (Giesecke, 1975; Giesecke & Van den Heever, 1981) suggest that stressful conditions of 1 udder quarter may be associated with CIR's in other apparently normal quarters of the same udder. The present investigation supports the earlier findings. It shows conclusively that CIR's did occur in lactating cows (Table 3–7). The CIR's where characterized by low-level fluctuations within the range of normal values (Table 2) of several lacteal parameters which showed slightly increased mean values and variability, and more significant correlations (Table 6) than under normal conditions (Table 2).

The fluctuating lacteal levels of the parameters monitored indicate an extensive range of intramammary adjustments in response to some factor(s) eliciting the CIR's. The adjustments of LAC, MAN, GAL and GLU were particularly pronounced (Table 3 & 4), preceded the fluctuations of SCC, BSA and NAG, and suggest regressive metabolic changes of pivotal importance to the function and integrity of the secretory epithelium and the re-adjustment of its lactational homeostasis.

The carbohydrate changes showed a pattern during the 24–168 h (Fig. 3 & 4) that almost paralleled the stepwise manifestation of regression in the unmilked udder halves (Giesecke *et al.*, 1990). This similarity suggests that milk stasis activated a general response in the cows. That response effected adjustments in both udder halves of epithelial carbohydrate metabolism, which were similar in nature but differed in magnitude, depending on the presence of the milk stasis perturbation in the unmilked and its absence in the milked udder quarters. The general response of the cows apparently superimposed certain changes of epithelial metabolism on the prevailing intramammary conditions. The CIR's observed in the normally-milked udder quarters were associated primarily with the general response of the cows to the milk stasis in the R quarters.

This association of CIR's, general response and epithelial metabolism requires further discussion from 3 main standpoints, namely—

* milk stasis as a stressful condition in the R quarters;
* spreading of CIR-promoting stimuli from the perturbed R to the normally-milked L udder quarters; and
* effects of such CIR-promoting stimuli on the secretory epithelium of the L udder quarters.

As regards the milk stasis as an intramammary irritant directly affecting the tissues of the R udder halves, data on stress (Giesecke, 1985a) suggest that the induced milk stasis amounted to a stressor. Its irritating effect on udder tissue apparently depended initially on composition, mass and pressure of the accumulating milk (Giesecke *et al.,* 1990). The unmilked quarters therefore showed a range of transitional re-adjustments amounting to a recognition response, alarm reaction and manifestations of regression (Giesecke *et al.*, 1990) (Fig. 5).

With regard to the spreading of CIR-promoting stimuli from the irritated R to the non-irritated L udder quarters, it seems unlikely that the cellular biochemical mediators, of immediate significance to the recognition response in the unmilked R udder quarters (Giesecke *et al.*, 1990), also directly initiated the CIR's in the milked L quarters (Table 3 & 4).

Although there are possible veinous anastomoses (Linzell, 1974), there is a separate arterial blood supply to each udder half and its quarters (Ziegler & Mosimann, 1960). The transmission of the mediators from R to L quarters through the vascular system therefore seems improbable from several points of view, such as dilution, inactivation and degradation of the mediators.

However, such limitations are not applicable to neuro-endocrinological stimulation to the bovine udder (Fig. 5) whose innervation consists of sensory

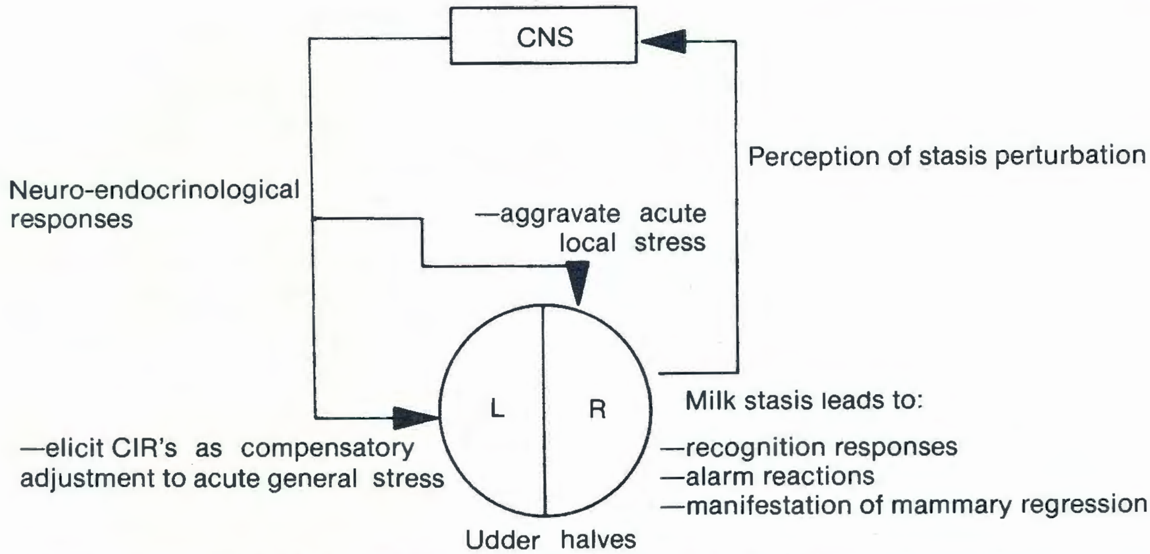

FIG. 5 Proposed model of general and intramammary major effects of escalating milk stasis induced in R udder halves and associated with CIR's in the L udder halves

and sympathetic vasomotor fibres. The former act as an afferent path to the CNS (Fig. 5), and the latter as an efferent path for direct transmission of impulses from the CNS to the effectors (e.g. smooth muscle fibres surrounding arteries, teat canals, teats and portions of the large lactiferous ducts). The network of sensory and sympathetic fibres is extensive, accompanies the arterial vessels down to the smallest arterioles and is exquisitely sensitive (Ziegler & Mosimann, 1960; Linzell, 1974).

The sympathetic-adrenomedullar system responds readily to various stressors (Giesecke, 1985a). It is thus the most likely channel for the transmission of signals from the perturbed to the unperturbed udder halves. Furthermore the lactating udder is highly sensitive to adrenalin (Linzell, 1974).

Because of its high metabolic requirements, its innervation, and its responsiveness to elevated sympathetic-adrenal stimulation the lactating bovine udder is particularly sensitive to various stressors (Giesecke, 1985a; Giesecke *et al.*, 1988) which promote epithelial regression (Giesecke, 1985 a, b). Mild stress and fasting reduce mammary blood flow (Linzell, 1974). However, this does not preclude the transient vasodilatory activities in quarters directly affected by the initial stages of milk stasis proposed by Giesecke *et al.* (1990).

As regards the effects of the CIR-promoting stimuli in the milked quarters, it is conceivable from the data above (Fig. 1–5) that the milk stasis in the unmilked quarters was initially perceived as a stressor-impulse by sensory receptors of the mammary innervation, and eventually transmitted to the general control system of stress (Giesecke *et al.*, 1988). That control centre of the central nervous system (CNS, Fig. 5) apparently activated the appropriate neuro-endocrinological emergency response by stimulating sympathetic-adrenomedullar activities to facilitate increased secretion of catecholamine(s) (Fig. 5). The latter affected certain enzymes and controlled metabolic changes that were associated with the fluctuating carbohydrate levels determined in milk (Fig. 3–5). The fluctuating levels of GLU (Fig. 3) and other reducing sugars (Table 3–5) support the proposed model of the development of CIR's (Fig. 5). It suggests that: (i) the milk stasis induced in the unmilked quarters, was perceived as intramammary stressor which stimulated the cow's control system of stress to elicit a form of acute general stress; (ii) the general stress affected the lactating udder as a whole, thus aggravating conditions related to the acute local stress in the R quarters, as well as initiating CIR's in the normally-milked L quarters; (iii) the CIR's amounted to compensatory adjustments to the acute general stress because regular milking protected the L quarters from stressful effects similar to those of the milk statis perturbation in the R quarters.

The data (Fig. 3 & 5) imply that local and general stress affect lacteal GLU concentrations in particular. GLU was the only parameter showing significant to highly significant correlations with each of the other parameters monitored (Table 6). It is thus apparent that stressful conditions and their effects on the glucose metabolism of the lactating udder are of pivotal importance to udder health.

General increases in adrenalin would elevate the availability of blood glucose. This could account for the escalations, during the initial 24 h of milk stasis, of the lacteal levels of LAC, MAN, GAL and GLU (Table 3–5) in the L quarters.

Elevated cortisol activities apparently inhibit prolactin-stimulated formation of PGE1 in the epithelial cell membrane (Horrobin, 1979). This has major consequences for the interactions of prolactin, cyclic AMP, cyclic GMP, intracellular Ca^{++} regulation and a range of epithelial functions (e.g. lactose and protein synthesis, milk secretion) and enzyme activities (e.g. adenylate cyclase, ATPases) (Giesecke *et al.*, 1988).

The combined effects of increased adrenalin and cortisol activities in the udder could therefore trigger major decreases of epithelial metabolism. Sudden reductions of LAC, MAN, GAL and GLU occurred between 24–48 h of milk stasis in the unmilked quarters (Giesecke *et al.*, 1990).

Corresponding values of LAC, MAN, GAL and GLU during the CIR's of the milked quarters (Table 4) showed total reductions over ranges covering 53,4 %, 77,6 %, 106,9 % and 102,5 % of baseline values, respectively. The decreases of LAC, MAN, GAL and GLU in milk of the milked udder halves are thus consistent with the view that acute general stress (Giesecke, 1985a; Giesecke *et al.*, 1988) could account for the CIR's of the L udder quarters (Table 3). Similar changes in the unmilked quarters with persisting milk stasis (Giesecke *et al.*, 1990), support the view that effects related to acute general stress were superimposed on epithelial alterations induced directly by milk stasis (Fig. 5). Consequently, acute general stress aggravated acute local stress and amplified the changes to levels seen in milk from the unmilked quarters (Giesecke *et al.*, 1990).

The metabolic fluctuations of CIR's coincided with slight re-adjustments of SCC, BSA and, in particular, of NAG (Table 3), indicating changes of epithelial integrity, permeability to BSA and lysosomal enzyme activities.

It is obvious from the low mean values and limited variations in SCC's (Table 2–3), and from their negative correlations with MAN, GAL and GLU values (Table 6), that decreases in the sugars were associated with increases in cellular wear and tear.

NAG showed similar correlations with MAN and GAL, whereas its correlation with GLU was highly negative ($P<0{,}001$) (Table 6). Such data imply that under CIR conditions the reductions of GLU, in particular, stimulated NAG activities, and increased cellular wear and tear to levels which, though increased, were nevertheless too slight to affect significantly the epithelial permeability to BSA. However, irrespective of such slight fluctuations, the significant correlations of GLU with each of the other parameters (Table 6) emphasize the pivotal importance of GLU to epithelial metabolism, integrity, permeability and lysosomal enzyme activities.

Because of its close correlation with elevated SCC values and several other lacteal parameters, NAG has been proposed by Obara & Komatsu (1984), Sandholm & Mattila (1985, 1986a, b), and Kangasniemi, Pelkonen & Tanhuanpää (1986), as a parameter for the diagnosis of subclinical mastitis. This is further supported by data on NAG as a lysosomal enzyme associated with leucocytes (Dingle, 1977), deteriorating integrity of mammary epithelium (Kitchen, Middleton & Salmon, 1978; Obara & Komatsu, 1984), and the stepwise degradation of hyaluronic acid by hyaluronidase, *beta*-glucuronidase and NAG (Pugh, Leaback & Walker, 1957).

However, from the point of view of the comparitively low mean SCC values under CIR conditions (Table 3) one should appreciate that NAG occurs also in milk from non-infected udders (Sandholm & Mattila, 1986a), in different subfractions of milk (Mellors, 1968; Kitchen *et al.*, 1978), and in microsomal, mitochondrial and nuclear fractions of mammary tissue (Weismann, Rowin, Marshall & Friederici, 1967; Kitchen *et al.*, 1978).

The correlations (Table 6) of NAG with other parameters indicate that, under CIR conditions monitored during this investigation, neither the SCC levels nor NAG activities were correlated significantly with BSA, as a marker of paracellular epithelial permeability to serum proteins and corresponding deteriorations of the epithelial junctional complex.

Furthermore, it should be noted that, under CIR conditions, neither SCC, BSA nor NAG showed significant negative correlations with LAC (Table 7), as is usually the case under more serious regressive and mastitic circumstances. It is apparent therefore that synthesis of LAC was not significantly affected by the changes of SCC, BSA and NAG (Table 3).

On the other hand, fluctuations of NAG were negatively correlated with MAN, GAL and GLU (Table 6) at levels of significance ranging from $P<0{,}01$ to $P<0{,}001$. This suggests that, under the CIR conditions monitored, the increased NAG activity showed highly, to very highly, significant correlations with the reduced epithelial metabolism of MAN, GAL and, especially, of GLU.

It is known than NAG splits *beta*-glycosides of both N-acetylglucosamine and N-acetylgalactosamine (Werries, Wollek, Gottshalk & Buddecke, 1969; Dingle, 1977). NAG thus hydrolyzes UDP-N-acetylglucosamine (Werries *et al.*, 1969) and UDP-N-acetylgalactosamine (Dingle, 1977). These are intermediates in the biosynthesis of N-acetylneuraminic acid, which is essential to the synthesis of oligosaccharides and glycoproteins (Diem & Lentner, 1970). The former are, for example, of vital importance to cellular membranes, whereas the latter include proteins, such as casein, *alpha*-lactalbumin, *beta*-lactoglobulin (Giesecke *et al.*, 1990) and lactoferrin (Nonnecke & Smith, 1984).

The correlations of NAG, MAN, GAL and GLU (Table 6) therefore imply that the increase in NAG was a response to a reduction of carbohydrates as a source of carbon for glycoprotein synthesis, and was therefore a symptom of changed epithelial synthesis of oligosaccharides and glycoproteins. Data on proteins in regressive and mastitic udder secretions (Giesecke, 1985b; Giesecke *et al.*, 1990; Harmon, Schanbacher, Ferguson & Smith, 1976; Smith & Schanbacher, 1977; Nonnecke & Smith, 1984; Thomas & Fell, 1985) indicate that under CIR conditions the main changes of glycoprotein synthesis could involve decreases of casein and increases of lactoferrin production.

Whether under such circumstances the lactoferrin would improve antibacterial properties of milk (Harmon *et al.*, 1976; Smith & Schanbacher, 1977; Nonnecke & Smith, 1984; Thomas & Fell, 1985) or whether other changes (Table 4) would stimulate bacterial growth, as observed in mastitic whey by Sandholm & Mattila (1985), Mattila *et al.* (1986) and Kaartinen & Sandholm (1986), remains unclear. In contrast, it becomes apparent from the present investigation that CIR's occurring as a result of stress, affect several aspects of the epithelial carbohydrate metabolism and could disturb the natural defence of the udder against bacterial infection.

Conclusion

From the few references applicable to CIR's in lactating bovine udders it is concluded that knowledge on the pevalence, diagnostic characteristics and udder health implications of CIR's is almost non-existent.

This investigation has shown that CIR's do occur. They develop when stressful secretory disturbances of one quarter lead to corresponding changes in other quarters of the same udder.

Compared with normal baseline values, the parameters monitored under CIR conditions showed comparatively slight increases of mean values and variability. The CIR's were associated with initial fluctuations in milk of GLU and of other sugars, followed by changes of NAG, BSA and SCC. This emphasizes the susceptibility of lactating udders to conditions affecting their glucose metabolism.

The re-adjustments under CIR conditions of LAC, MAN, GAL and GLU are consistent with: (i) the function of the general control system of stress,

(ii) initial triggering of a provisional, primary alarm reaction by means of increased sympathetic-adrenomedullar activities, (iii) subsequent triggering of a more profound secondary alarm reaction by means of increased sympathetic-adrenomedullar and -adrenocortical activities, and (iv) effects of mild acute general stress on healthy lactating mammary epithelium milked normally.

Such re-adjustments revolved around the epithelial glucose metabolism. The pivotal role of glucose is supported conclusively by the fact that GLU was the only parameter correlated significantly with each of the other parameters monitored under CIR conditions.

The results from this and the parallel investigation on aspects of the CIR's and mammary regression respectively indicate that complete milk stasis, as a stressor directly affecting unmilked udder quarters, provoked a certain degree of acute general stress. This supplemented and aggravated the epithelial changes already existing in the disturbed unmilked udder quarters and elicited corresponding new changes, i.e. CIR's, in the normally-milked quarters that were, to a large extent protected, by normal milking.

The development of CIR's, as intramammary adjustments to general stress, and its implications for healthy udder quarters milked normally, as well as for quarters perturbed by various local stressors, require further investigation.

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