Changes in N-Acetyl-β-D-Glucosaminidase Activities in Relation to Other Milk Components Throughout Normal Lactation in the Guinea Pig

E. ROETS, C. BURVENICH, and G. VANDEPUTTE-VAN MESSOM
Department of Veterinary Physiology
Veterinary Faculty of the University of Ghent
Casinoplein 24, B-9000 Gent, Belgium

ABSTRACT

Changes in milk production, NAGase activities, Na, Cl, K, lactose, total N, and fat were followed throughout normal lactation in 26 healthy guinea pigs. Appropriate microassays were used and all determinations were performed on the same small skim milk sample (microhematocrit tube). A very sensitive and rapid spectrofluorimetric determination of NAGase in 10-μl skim milk samples was evaluated.

Four days after parturition, daily milk production reached a maximum of about 20 ml. Thereafter milk yield decreased progressively to about 7 ml on d 15. During the entire course of lactation, a progressive increase in NAGase, total N, fat, Na, and Cl was observed. However, K and lactose decreased. Highly significant positive correlations between NAGase and Na, Cl, fat, and total N were evident, whereas correlations were negative between NAGase and K, lactose, and milk production. These changes can be explained on basis of alterations that occur at the level of the blood-milk barrier. The short lactation period of the guinea pig, the easy handling and milking practices, together with the available sensitive micromethods can lead to the establishment of a convenient guinea pig mastitis model.

INTRODUCTION

The guinea pig has been chosen by several authors (11, 14) as a suitable model for studying the physiology of normal milk secretion during an entire lactation. A more extensive study with an excellent analysis of milk production, macroingredients, and macrominerals has been performed recently (1, 2, 3, 16). In these studies during lactation, milk K and lactose decreased, whereas Na and Cl increased. These changes, normally occurring after about d 6 of lactation, are attributed to an increase of the paracellular movement of ions and small molecules between extracellular fluid and milk (13). This is in agreement with the changes observed in milk during late lactation in the cow. The guinea pig has also been used to study leukocyte migration in the milk during experimentally induced endotoxin mastitis (9, 10).

Concomitant changes in the concentrations of some milk components are typical for the diagnosis of bovine mastitis, i.e., a decrease of lactose and K and an increase of Na, Cl, and serum albumin concentration. An influx of polymorphonuclear blood cells into milk occurs as well (18). It appears that of the different enzymes secreted into the milk, the lysosomal enzyme NAGase is a good parameter for the diagnosis of mastitis. Significant correlations between NAGase activity and SCC (6, 7), Cl, lactose, and lactoferrin (12) have been demonstrated in cow's milk. Moreover, NAGase activity was indicative of the severity of inflammation of the udder.

The establishment of a guinea pig mastitis model, in which determination of milk production and composition by daily milking is performed, could be very useful for preliminary studies of the inflammation of the mammary gland. Experimentally induced mastitis experiments on lactating cows are very expensive and time-consuming.

The objective of the present work was to study the changes in NAGase activities in relation to other components in milk throughout normal lactation in guinea pigs. All determinations were performed on the same milk sample

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collected during the entire lactation period (15 d). Furthermore, the possibility to use a very sensitive (10 μl skim milk) and rapid spectrofluorimetric determination of NAGase as a marker of inflammation was evaluated.

MATERIALS AND METHODS

Animals

Healthy female common guinea pigs were mated (one male per group of 6 to 10 females). To avoid individual variability, experiments were performed during second lactation. Twenty-six guinea pigs at about 6 wk of age were used. The guinea pigs were ear-tagged and kept under controlled temperature (19 ± 1°C) in a box with straw on the floor. They were fed at regular times (0800 and 1600 h) with commercial guinea pig pellets, carrots, cabbage, and grass. This diet was regularly supplemented with ascorbic acid in the drinking water, which was provided for ad libitum intake. The weight of the mothers on the day of the second parturition was 944 ± 52 g (mean ± SE).

Collection of Milk

In order to ensure the presence of sufficient milk in the mammary glands, the mothers were separated from the litter for 6 h before milking. Milking occurred daily during the lactation period. Although the use of oxytocin during milking has been recommended in literature (8), we did not use it because: 1) the injections induce different degrees of stress, 2) oxytocin may alter milk electrolyte composition, and 3) only minor differences in the milk volume obtained with or without oxytocin, were observed in preliminary experiments. Milk was obtained at 1400 h by means of machine designed by McKenzie and Anderson (8). The guinea pig was placed on its back in the operator's hand and arm. By gentle massage of the glands and under a slight negative pressure (20 ± cm Hg), milk was collected in preweighed plastic vials. Milk from each of the two mammary glands of an animal was sampled separately. After being weighed both milk samples were mixed for further analysis. Because the rate of milk secretion is constant, daily milk production (MP) (g/24 h) was calculated by multiplying the amount of milk produced in 6 h (between 0800 and 1400 h) by 4.

Chemical Determinations

Because guinea pig milk contains high amounts of fat (>6%), and fat sometimes influences some chemical determinations, we decided to determine the different milk constituents in fat-free milk. Fresh milk (about 70 μl) was collected directly into capillary tubes of 75-mm long, sealed, and centrifugated at 4°C for 15 min at 12,000 × g in a microhematocrit centrifuge (Hawksley Ltd., Lancing, Sussex). After centrifugation, the length of the column of fat, which was clearly separated from the skim milk, was measured and calculated as a percentage of the total volume of milk in the tube. To collect the skim milk, the upper part of the capillary tube just below the boundary of the packed fat was cut off, and appropriate volumes (10 μl for NAGase, K and Na; 20 μl for lactose and Cl; 100 μl for total N) were obtained with a 50-μl microsyringe and stored at −20°C until analysis.

Sodium and K were estimated by means of flame photometry; Cl was measured with a chloride meter. Lactose was assayed without deproteinization according to Yamashita and Watanabe (19), adapted for guinea pig milk by Mepham and Beck (11). Total N (TN) was determined by Kjeldahl.

Determination of N-Acetyl-β-D-Glucosaminidase and Milk Components

The lysosomal enzyme NAGase was measured in skim milk with a fluorimetric procedure using 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide as substrate. Skim milk was used, because in cow's milk, 91% of the total activity is present in the skim milk fraction (7). Moreover, when using whole milk, turbidity problems were often encountered and spectrofluorimetric readings were inaccurate. These problems, which are probably due to the high amounts of fat in guinea pig milk, could not be overcome by using a small volume (10 μl) of fresh milk or by improving fat dispersion by incorporating a detergent (.1% Triton X-100) in the buffer solution.

The enzymatic reaction was started by adding 10μl of skim milk to a tube containing 200

TABLE 1. Concentrations and relative changes of Na, K. Cl, lactose, fat, NAGase, and total N in guinea pig milk during second lactation.

Parameter	Mean	SEM	Range	Curve course
Na, m*M*	20.8	1.6	13.8–33.8	Increase
K, m*M*	23.4	1.3	12.0–28.0	Decrease
Cl, m*M*	45.5	4.5	24.5–79.0	Increase
Lactose, g/100 g	4.3	.2	2.9–5.1	Decrease
Fat, %	11.0	.6	8.0–15.8	Increase
NAGase, n*M*/min per ml	20.6	1.9	11.2–38.7	Increase
Total N, mg/100 mg	767	40	631–1074	Increase

μl of 2 m*M* 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide in .25 *M* citrate buffer (pH 4.4), and incubated for 10 min at 37°C. The reaction was terminated by the addition of 5.5 ml of .1 *M* carbonate buffer (pH 10), and the released 4-methylumbelliferone was measured at an excitation wavelength of 365 nm and an emission wavelength of 450 nm, using a spectrofluoriphotometer. Enzyme activity was expressed as nanomoles of liberated 4-methylumbelliferone per minute in 1 ml of skim milk.

RESULTS

In Table 1, data of the different milk components during the 15 successive d of lactation were averaged for all animals (n = 26). With the exception of K and lactose, all milk components analyzed increased in concentration progressively during lactation, differing by about a factor two to three times between start and finish.

The mean daily average of the twenty-six animals for MP and NAGase are shown in Figure 1. Daily MP varied among animals and ranged from 5.9 to 18.9 g (mean of 11.8 ± 1.2 g/24 h). The MP increased rapidly until d 4 and then declined slowly until d 10. During the last part of the lactation, MP was low and amounted to about 7 g. The NAGase activities decreased from 23.5 ± 1.5 n*M*/min per ml on d 1 to 11.2 ± .9 n*M*/min per ml on d 4 and then increased steadily to 38.7 ± 2.6 n*M*/min per ml on d 15.

Mean changes in the concentrations of fat and TN and also shown in Figure 1. The changes in Na, K, Cl, and lactose are in Figure 2. At the onset of lactation, average lactose and fat concentrations were 4.8 ± .2 and 10.9 ± .8 g/100 g, respectively. At the end of lactation (d 15) these parameters averaged 2.9 ± .5 and 15.8 ± 1.6 g/100 g. Total N decreased from 823 ± 62 mg/100 ml on d 1 to 624 ± 52 mg% on d 6 and then increased steadily to 1031 ± 79 mg/100 ml on d 15; Na and Cl showed a similar pattern. At the onset of lactation, Na and Cl concentrations were 14.7 ± 1.0 and 24.5 ± 1.3 m*M*, respectively, whereas by d 15 they were 33.8 ± 5.0 and 79.0 ± 5.4 m*M*. Potassium fell continuously during lactation from 28.0 ± .1 to 12.0 ± 5.0 m*M* (d 15).

In order to investigate whether secretion of the different milk components are subjected to a common mechanism of control, correlation coefficients between the different pairs of parameters were calculated (Table 2). Highly positive or negative correlation coefficients ($P<.001$) observed between all parameters demonstrate a linear relationship of their concentrations to each other.

DISCUSSION

Changes in MP, NAGase activities, and Cl, Na, K, lactose, fat, and TN concentrations were investigated throughout the second lactation in 26 healthy guinea pigs. Using micromethods and fat-free milk, all these analyses could easily be performed on about 250 μl of milk. Apart from the Kjeldahl N determination, the different analyses were done on microamounts of skim milk (10 to 20 μl). As no deproteinization of the samples is required and stable reagents were used, these analyses were quick and rather inexpensive. Significant changes in MP, Cl, Na, K, lactose, and fat concentrations during lactation were observed and the data are in agreement with those from literature (1, 2, 3). In these lactation studies, during established lactation (d 1 to about d 7), milk composition remains nearly constant, indicating the presence

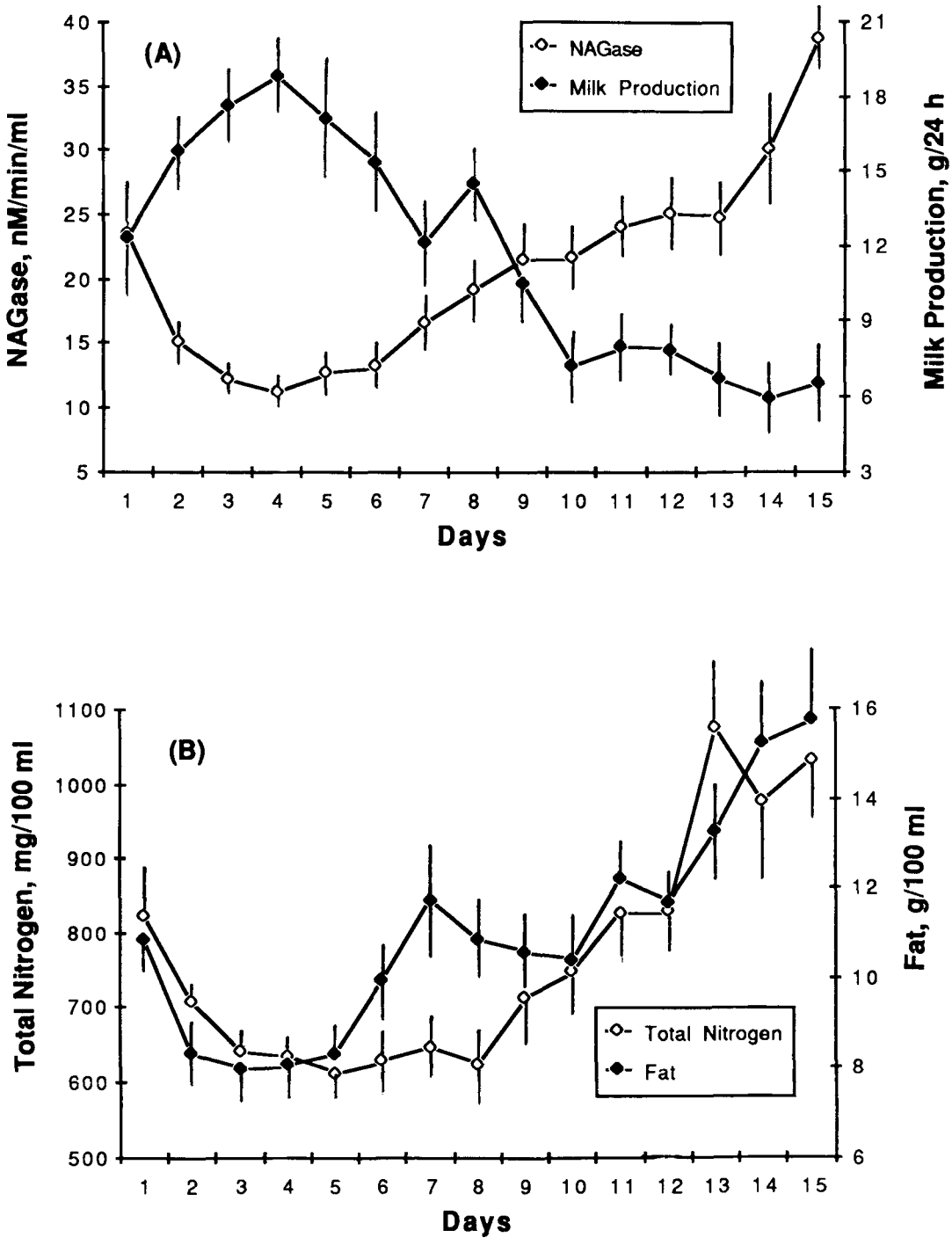

Figure 1. Mean and SEM for milk production and concentrations of NAGase, (A), total N, and fat (B) throughout lactation in 26 guinea pig sows.

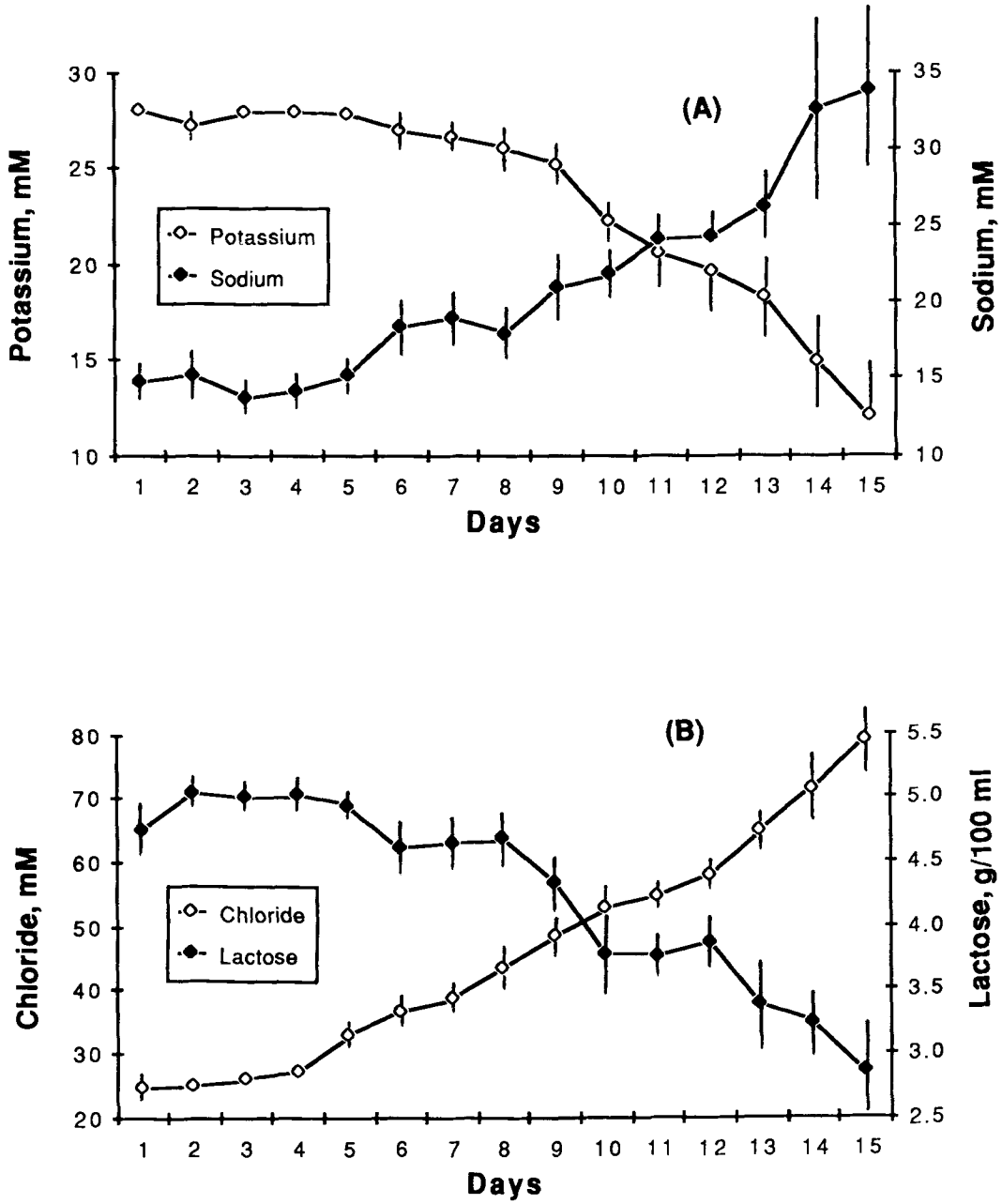

Figure 2. Mean data and SEM for concentrations of K and Na (A), Cl and lactose (B) throughout lactation in 26 guinea pig sows.

of a "tight" blood-milk barrier in the mammary gland of these animals.

In goats (4) and cows (17) hematocrit readings of fat content in milk were overestimated by about 20% as compared with classical methods. Due to the small amount of milk collected in our experiments, the Gerber method (sample volume: 11 ml milk) could not be compared

TABLE 2. Correlation coefficient for pairs of parameters collected from 26 guinea pigs during 15 consecutive d. All values were highly significant ($P<.001$).

Parameter	Na	Cl	Lactose	Fat	Total N	NAGase	Milk yield
K	−.98	−.96	.97	−.90	−.89	−.91	.86
Na		.98	−.97	.94	.86	.90	−.88
Cl			−.98	.90	.79	.87	−.88
Lactose				−.91	−.79	−.90	.93
Fat					.82	.92	−.87
Total N						.86	−.82
NAGase							−.87

with results obtained by hematocrit centrifugation. It was found, however, that milk fat values measured with both methods were highly correlated (4, 17).

The NAGase activity of guinea pig's milk has not been determined by others. In comparison with all other parameters, the change in concentration from the start to the end of lactation was most striking with NAGase (fourfold increase). As correlations with the other milk components were highly significant (Table 2), determination of the enzyme seems to be a good and suitable parameter for estimating changes in secretion of milk of guinea pigs. These correlations were also found in milk of cows, some of which were affected by mastitis (6, 7, 12). With the exception of d 1, normal NAGase activities (d 2 to 6) amounted to about 13 n*M*/min per ml and were twice as high as the activities measured in normal quarter milk of the cow [2.9 (15); 5.1 (7); and 5.3 n*M*/min per ml (5)]. The high NAGase activity (23 n*M*/min per ml) noted on d 1, together with the elevated concentrations of TN and fat in milk, are possibly due to the secretion of colostrum.

Because correlations were good between SCC and NAGase activities in cows, the determination of the enzyme in milk reflects the number of cells in milk. At the end of lactation, a disintegration of the blood-milk barrier occurs, and an increase of the paracellular secretion therefore results in an increase of Na and Cl and a decrease of K and lactose in milk (13). During that phenomenon, SCC increases, probably from the influx of typical blood proteins into the mammary gland. It is therefore not surprising that correlations between NAGase activities and concentrations of electrolytes in milk were observed.

Due to the very short lactation period of the guinea pig and the easy handling and milking practices of the animals, several experiments can be done at once. These advantages and the availability of sensitive micro-methods to evaluate changes in milk composition can lead to the establishment of a convenient guinea pig mastitis model.

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REFERENCES

1 Anderson, R. R., M. S. Salah, L. G. Sheffield, and W. N. McKenzie, Jr. 1984. Milk production and lactation curve of guinea pigs. J. Dairy Sci. 67:185.
2 Anderson, R. R., and D. D. Chavis. 1986. Changes in macroingredients of guinea pig milk through lactation. J. Dairy Sci. 69:2268.
3 Anderson, R. R., and L. G. Sheffield. 1988. Macrominerals in guinea pig milk during 21 days of lactation. J. Dairy Sci. 71:337.
4 Fleet, I. R., and J. L. Linzell. 1964. A rapid method of estimating fat in very small quantities of milk. J. Physiol. (Lond.) 175:15P.
5 Kitchen, B. J., and G. Middleton. 1976. Enzymatic methods for the estimation of the somatic cell count in bovine milk. J. Dairy Res. 43:491.
6 Kitchen, B. J., G. Middleton, and M. Salmon. 1978. Bovine milk N-acetyl-β-glucosaminidase activity and its significance in the detection of abnormal udders secretions. J. Dairy Res. 45:15.
7 Kunkel, J. R., R. B. Bushnell, J. Cullor, and J. Aleong. 1987. Studies on induced *Klebsiella* mastitis with relationships among N-acetyl-β-D-glucosaminidase, bacterial and somatic cell counts. Cornell Vet. 77:225.
8 McKenzie, W. N., Jr., and R. R. Anderson. 1979. A modified device for collecting milk from guinea pigs. J. Dairy Sci. 62:1469.
9 McKenzie, W. N., Jr., and R. R. Anderson. 1981. Endotoxin induced migration of leukocytes from blood to milk. J. Dairy Sci. 64:227.
10 McKenzie, W.N., Jr., and R. R. Anderson. 1981. Endo-

toxin dose-related leukocytosis in milk of guinea pigs. J. Dairy Sci. 64:2234.
11 Mepham, T. B., and N.F.G. Beck. 1973. Variation in the yield and composition of milk throughout lactation in the guinea pig (*Cavia porcellus*). Comp. Biochem. Physiol. 45:273.
12 Obara, Y., and M. Komatsu. 1984. Relationship between N-acetyl-β-D-glucosaminidase activity and cell count, lactose, chloride, or lactoferrin in cow milk. J. Dairy Res. 67:1043.
13 Peaker, M., and J. C. Taylor. 1975. Milk secretion in the rabbit:changes during lactation and the mechanism of ion transport. J. Physiol. (Lond.) 253:527.
14 Peaker, M., C.D.R. Jones, J. A. Goode, and J. L. Linzell. 1975. Changes in milk composition during lactation in the guinea-pig, and the effect of prolactin. J. Endocrinol. 67:307.
15 Pyörälä, S. 1987. Effect of some vehicles used in intramammary mastitis preparations on inflammation indicators in milk. J. Vet. Pharmacol. Ther. 10:248.
16 Sheffield, L. G., and R. R. Anderson. 1985. Relationship between milk production and mammary gland indices of guinea pigs. J. Dairy Sci. 68:638.
17 Vandeputte-Van Messom, G., and C. Burvenich. 1989. Comparison of fat and cream content in normal and mastitic milk of cows. Vet. Q. 11:61.
18 Vandeputte-Van Messom, G., C. Burvenich, E. Roets, and L. A. Devrieze. 1988. Effect of bovine somatotropin on milk yield and composition during *Escherichia coli* induced mastitis in lactating cows: some preliminary results. Vlaams Diergeneeskd. Tijdschr. 57:53.
19 Yamashita, M., and F. Watanabe. 1973. Determination of serum glucose with *o*-toluidine without glacial acetic acid. Clin. Chim. Acta 47:211.