A-Esterase Activities of Milk From Cows with Experimentally Induced Mastitis

R. R. MARQUARDT, T. L. FORSTER, G. R. SPENCER, and G. H. STABENFELDT
Department of Animal Sciences (Dairy) and Department of Veterinary Pathology
Washington State University, Pullman

Abstract

Variations in CMT (California Mastitis Test), cell count, catalase, chloride, and A-esterase levels following the inoculation of normal lactating quarters of two cows with varying numbers of αβ hemolytic staphylococci (previously isolated from a cow with mastitis) are described. Since the organism did not produce as strong a response as desired, the quarters were rein-}

Infusions of sterile broth into the two control quarters of Cows 506 and 583 did not affect A-esterase levels. Infusions of varying levels of αβ hemolytic staphylococci into the three remaining quarters of each of the two cows generally resulted in a marked increase in A-esterase activity within a 12-hr period, followed by a gradual decline. Similar patterns of change were also observed for catalase and cell counts. Coefficients of correlation between A-esterase and the four other tests were: A-esterase × CMT = 0.69, n = 192 individual samples tested; A-esterase × cell counts = 0.78, n = 188; A-esterase × catalase = 0.80, n = 192; and A-esterase × chloride = 0.60, n = 192.

Aldridge (1) described in 1953 the existence of an A-esterase in mammalian blood sera. In 1959, after a comprehensive survey of the plasma of vertebrates for A-esterase, Augustinsson (2) proposed that A-esterase include those enzymes resistant to organophosphorous compounds and catalyze the hydrolysis of aromatic esters such as phenyl acetate. A more detailed report on some of the other properties of A-esterase has been given (3).

The presence of A-esterase in milk was first reported by Forster et al. (6). In a subsequent paper they observed that mastitic milk often had elevated A-esterase activities (7). The object of this study was to investigate the relationship between degree of mastitis and milk A-esterase levels. Preliminary results of these studies have been reported (9).

Experimental Procedures

Isolation and culturing of αβ hemolytic staphylococci. An isolate of a coagulase-positive Staphylococcus sp. (462C) was recovered from a cow with mastitis in the Washington State herd. This organism produced alpha and beta hemolysins on sheep blood agar. The isolate was grown in tryptose phosphate broth for 24 hr at 37 C (17).

Selection and infection of cows. Two Holstein heifers, 506 and 583, whose milk had low CMT scores, were selected. Three days after the experiment was initiated varying numbers of αβ hemolytic staphylococci were infused into three of the four quarters of each cow. Sterile broth was infused into the fourth uninfected quarter of each cow. This quarter served as a control. Since the virulence of the organism was not as great as expected, reinfections were made, to establish a more marked response. Dosage schedules for each quarter on the various infection days are presented in Table 1.

Collection and storage of milk samples. Foremilk samples (about 116 g) were collected in glass containers at either 12- or 24-hr intervals (see Figure 1, 2, or 3). The samples were placed in an ice-water bath and transported to the laboratory. They were stored in a refrigerator until analyzed. The maximum time interval between collecting and analysis was two days.

Analysis of milk. All milk samples were routinely examined for CMT reaction, cell count, catalase activity, and chloride. These values served to evaluate the degree of mastitis established by the experimental infection. They also served as a basis for evaluating the relationship between mastitis and A-esterase levels.
TABLE 1
Dosage schedules for Quarters A, B, C, and D* of Cows 506 and 583

<table>
<thead>
<tr>
<th>Day</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>13.7</td>
<td>6.8</td>
<td>27.4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>5.4</td>
<td>2.7</td>
<td>10.8</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>12.9</td>
<td>6.5</td>
<td>25.8</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>6.8</td>
<td>3.4</td>
<td>13.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Broth carrier (ml) 2 2 2 2
Dilution factor b 1:100 1:200 1:50

* A—right front, B—right rear, C—left rear, D—left front.

b Dilution of bacterial culture.

CMT reagent was obtained from Norden Laboratories, Inc., Forest Grove, Oregon, and the test was performed in the manner described by Schalm and Noorlander (14). Cell counts were estimated by procedures outlined by Spencer and Simon (16), and chloride measured by the Sanders modification of the Volhard titration (13).

The method of determining catalase activity was similar to that proposed by Spencer and Simon (16). However, in this study 15-ml graduated centrifuge tubes and rubber stoppers with 2-mm (inside)-diameter glass tube openings were substituted for screw-cap test tubes. One additional modification of the basic procedure involved the addition of one eyedropper measure of a kerosene solution of Sudan Red to the reaction flask (15). This was added after the milk and H₂O₂ were added, and facilitated final reading of oxygen evolution.

Milk A-esterase activity was determined by Warburg manometric techniques similar to those described by Forster et al. (7). The basic assay procedure, however, was modified to include only one set of duplicate blanks with each run, rather than with each individual milk sample. This change was made after observing that there was very little difference between blank values for various milk samples within the same run. Activity values were expressed as units (i.e., μmoles of substrate hydrolyzed per minute per milliliter of milk).

Results

Changes in A-esterase activity during the 17-day experimental period for each of the four quarters of each cow are presented in Figures 1 and 2. In Cow 583, the uninfected control quarter maintained essentially a constant A-esterase value throughout the entire experiment. The level of A-esterase in the three other quarters of Cow 583 remained uniform until infected, after which there was a sharp rise. This was followed by a gradual decline in activity in the three quarters to Day 7. The reinfection on Day 5 did not affect A-esterase levels. After the second reinfection on Day 8 there was a sharp rise in A-esterase activity, especially in the A quarter, where the activity was 1.99 units. These high values dropped sharply the next day, after which they tended to level off. The reinfection on Day 10 resulted in a slight increase in A-esterase activity for two of the three quarters. Following this final reinfection
the activity gradually declined and approached normal values. During the last three days of the experiment, A-esterase values in the three infected quarters tended to fluctuate. This corresponded with fluctuating CMT, cell count, chloride, and catalase values.

The pattern of change in A-esterase for Cow 506 was similar to that observed for 583. The magnitude of change, however, was not as large.

Results presented in Figure 3 give the average catalase, A-esterase, and cell count values for the six infected quarters of Cows 506 and 583. Averages of the two control quarters also are given. These results demonstrated that during the experimental period patterns of change in catalase, A-esterase, and cell count levels were similar. Positive correlations were obtained between the four commonly used mastitis tests and A-esterase activities. All of the samples tested in this study were used in computing the correlation coefficients. These coefficients were A-esterase × CMT = 0.69, n = 192 individual samples; A-esterase × cell count = 0.78, n = 188; A-esterase × catalase = 0.80, n = 192; and A-esterase × chloride = 0.60, n = 192.

Discussion

Results presented above demonstrate that under the experimental conditions described herein, where healthy quarters of first-calf heifers in early lactation were infected with αβ hemolytic staphylococci,
staphylococci, A-esterase activity is positively correlated with degree of mastitis as measured by CMT, cell count, catalase, and chloride. Booth et al. (4) have reported that following experimental exposure to *Streptococcus agalactiae*, a several-fold increase in A-esterase activity was observed and was closely associated with an increase in leucocyte numbers and blood serum albumin levels. These results, therefore, support the original proposal by Forster et al. (7), that A-esterase may serve as a factory screening test for mastitis. Other factors, however, such as stage of lactation (7, 12) and variations between cows in normal A-esterase levels (8) would tend to reduce the reliability of A-esterase as a screening test for mastitis. Preliminary reports (10, 11) have been made concerning the relationship between A-esterase in milk and the degree of mastitis. These reports have dealt with observations made on milk samples from a large number of individual quarters of many different cows, at various stages in the lactation cycle and from a number of different herds. Consequently, the factors mentioned above have contributed to the variations in A-esterase levels and, unquestionably, have influenced the correlation coefficients reported. More detailed results of these studies will be reported later.

The source of milk A-esterase has not been conclusively established. It has been hypothesized that A-esterase arises from the blood stream by diffusion of blood plasma across the mammary tissue (8). This hypothesis is supported by the observation that plasma has about 1,000 to 2,000 times as much A-esterase as normal milk (8). Results obtained in this study also support this theory. Lesions resulting from bacterial action in the udder may greatly increase the rate at which A-esterase diffuses across the mammary membrane, resulting in elevated milk A-esterase levels.

Our results indicated that the pattern of change in A-esterase activity following infection with aB hemolytic staphylococci was similar to that observed for catalase and cell counts. Perhaps if the cows in these experiments had been sampled and milked out (to eliminate dilution effects) at shorter intervals, a difference in the rate of appearance of the various blood components would have been shown.

Other researchers (5), using shorter collection periods, have demonstrated that there is a difference in the rate of appearance and rate of return to normal of various blood constituents in the mammary gland following induced mastitis. On the basis of these observations it was suggested that the exudation of various blood proteins involved at least two different processes. A study of changes in A-esterase levels, together with those of various other blood constituents, following experimentally induced mastitis may provide additional information on the mechanism by which various blood proteins are transported across the mammary tissue. Changes in A-esterase activity after an attack of mastitis may also provide an index of the degree that milk is altered in composition towards that of blood. Additional research should be carried out on these problems.

References


