# EFFECTS OF LEUCOCYTE DEGENERATION ON MASTITIS SCREENING TESTS[1]

B. SINGH[2] AND R. T. MARSHALL

*Dairy Department, University of Missouri, Columbia 65202*

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SUMMARY

Direct microscopic leucocyte counts (DMLC) and morphological studies indicated that leucocytes disintegrate and lysis progresses rapidly from day 1 to day 2 in milk samples during storage. The average DMLC/ml decreased 34% during the first two days of storage. The reactivity of the samples to the California Mastitis Test decreased with sample age. These combined observations suggest that deoxyribonuclease (DNA), once free of the intact leucocyte, becomes less reactive or non-reactive to the test reagents. Milk samples which produced more than 20% $0_2$ gave highly reproducible results when tested by the catalase method. Those which produced less than 20% gave erratic results.

A number of publications have indicated that changes take place in milk samples during storage which influence the results of mastitis screening tests made on the stored samples. Schalm and Noorlander *(5)*, Frank and Pounden *(2)*, and Tucker and Paape *(6)* each showed that the substance reactive to the California Mastitis Test (CMT) reagent became less reactive due to storage. Frank and Pounden *(2)* reported increased oxygen production in stored samples tested for catalase. However, Tucker and Paape *(6)* did not find a significant change in catalase content with storage up to four days. Nageswararao, Blobel and Derbyshire *(3)* found the catalase test unaffected by storage up to three days unless there was appreciable bacterial growth. No information was available regarding changes in direct microscopic leucocyte counts (DMLC) with time of storage.

Our experiments have been designed to verify certain reported observations and to seek reasons for the results. Stability of the reactive substance in the milk, preventing production of more reactive substance, and assurance that all of the substance is available to react would appear to be of prime importance.

## MATERIALS AND METHODS

Milk samples were collected aseptically from individual quarters of cows showing various degrees of reactivity to the California Mastitis Test (CMT) which was performed on foremilk immediately prior to sampling. Samples were immediately cooled at 4 C and held thereat except for the few minutes each day when aliquots were being removed for testing. Fourteen samples were collected. An additional 10 samples were later subjected to part of the tests.

Screening tests were performed initially and daily thereafter for five days. Methods used for the catalase test, the DMLC and the CMT were as described in Public Health Service Publication No. 1306 *(4)*. Bacterial counts were made as described in Standard Methods *(1)*. Reactions to the CMT were scored 1, 2, 3, 4 rather than T, 1, 2, 3 so that numerical averages could be calculated. Studies on morphological changes of leucocytes contained in milk were made during the first and second days after collection using phase-contrast and light microscopes.

## RESULTS AND DISCUSSION

The majority of the samples used in these experiments contained relatively low numbers of leucocytes. However, they are thought to be representative of an equal number of bulk herd milk samples.

TABLE 1. AVERAGE CMT SCORE PER DAY DURING STORAGE COMPARED TO ORIGINAL SCORE

| Number of samples Per group | Days of storage | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 |
| 6 | 1 | 0.7 | 0.2 | 0.2 | 0 | 0 |
| 6 | 2 | 1.5 | 1.2 | 1.0 | 0.5 | 0.3 |
| 1 | 3 | 3.0 | 2.0 | 3.0 | 2.0 | 1.0 |
| 1 | 4 | 4.0 | 3.0 | 2.0 | 2.0 | 2.0 |
| Mean | 1.8 | 1.4 | 0.9 | 0.9 | 0.5 | 0.4 |

Table 1 shows the changes in average CMT scores with storage time. The results indicate a somewhat faster rate of decline in score than observed by Tucker and Paape *(6)*. However, this may be due to the fact that we were observing fewer numbers of leucocytes initially. The stronger CMT reactions involve millions of leucocytes; whereas, the lower ones may involve only a few hundred-thousands per milliliter. Our results indicate that the lesser reacting samples decline faster in reactivity when a system of daily averages is used.

Analyses of the same samples by DMLC (Table 2) helped to explain the loss in CMT reactivity. A daily decrease in leucocyte counts was observed for each sample. The greatest decrease occurred between

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[2]Present Address: University of Pittsburgh, Graduate School of Public Health.

TABLE 2. AVERAGE DMLC PER DAY DURING STORAGE COMPARED TO ORIGINAL COUNT (GROUPED DATA)

| Count range of group (thous.) | Number of samples/group | Days of storage 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| 10-100 | 4 | 44[a] | 43 | 18 | 12 | 10 | 9 |
| 101-500 | 7 | 160 | 130 | 79 | 41 | 12 | 9 |
| 501-2000 | 2 | 1600 | 1400 | 950 | 550 | 420 | 110 |
| 19,000 | 1 | 19,000 | 18,000 | 13,000 | 12,000 | 12,000 | 8,600 |
| Mean | 14 | 1,680 | 1,560 | 1,110 | 960 | 930 | 640 |

[a]Counts expressed as thousands per milliliter

days one and two of storage for samples in each count range. Decreases averaged about one-third after two days storage. Numbers declined at a faster rate among samples containing the lower number of cells. The point of most importance is that countable numbers of leucocytes in 2-day old milk can be expected to have decreased by more than 34 percent. Therefore, leucocyte counts for most bulk samples would be expected to have decreased an intermediate amount, since some of the milk would be two days old while some would be relatively fresh. Based on our data a decrease of 20% would seem to be a realistic estimate. These observations are in contrast to those of Nageswararao, Blobel and Derbyshire (3) who reported that leucocyte counts remained constant for at least three days, but the proportion of live leucocytes decreased rapidly. Figure 1 shows photomicrographs of leucocytes taken during the first and second days after collection. The leucocytes had enlarged in size by the second day of storage. Observations using phase-contrast lenses indicated that the spherical nuclei did not have their usual lobed appearance. The swollen state, vacuolization of cytoplasm, deformity and altered staining of the nucleus suggested progressive lysis and disintegration of the leucocytes.

Data shown in Table 3 suggest that difficulties may arise in securing day-to-day uniformity of catalase test results. There were statistically significant differences between mean test values for days of storage in the initial experiment. Means for days 0, 1 and 4 were not significantly different, but means for days 2, 3 and 5 were significantly higher ($P < 0.05$). The differences were small in magnitude in relation to the current interpretation of the test, wherein classes of milk are based on ten percent-wide ranges. It was expected that small daily increases would be observed due to catalase production by bacteria. The high results for day five may reflect bacterial growth. Standard plate counts run on days 0, 2, 4 and 5 indicated bacterial populations of more than 1,000,000/ml in two samples on day 5. The highest count on day 4 was 570,000/ml and the average was 190,000/ml.

A second series of 10 samples was tested by the catalase method. Averages of these observations are shown in the last line of Table 3. A similar pattern of test results was observed. However, mean differences were smaller.

Examination of the individual results indicated that the samples with the lower initial oxygen production caused the increases at the 2 and 3-day storage times. When the samples were divided into two groups, those producing less than and those produc-

TABLE 3. AVERAGE PERCENT OXYGEN PER DAY DURING STORAGE COMPARED TO INITIAL RESULT USING GROUPED DATA

| % $O_2$ per group | Number of samples/group | Days of storage 0 | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|
| 0-10 | 5 | 7[a] | 9 | 12 | 10 | 8 | 13 |
| 11-20 | 3 | 15 | 19 | 22 | 18 | 15 | 19 |
| 21-30 | 3 | 26 | 29 | 30 | 29 | 27 | 33 |
| 31-50 | 3 | 39 | 37 | 37 | 36 | 33 | 40 |
| Mean | 14 | 19.0 | 21.0 | 23.5 | 21.5 | 19 | 24.5 |
| Second Series[b] | 10 | 23.3 | 24.1 | | 25.1 | 24.6 | |

[a]Percent oxygen

[b]Ten samples tested to confirm findings of first series. Individual results not included in table.

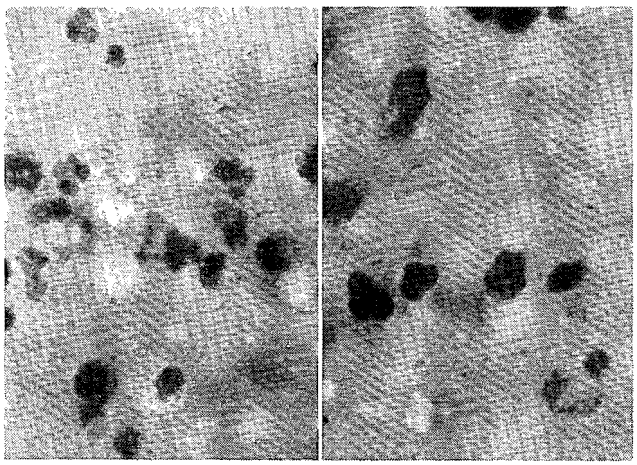

Figure 1. Photomicrographs of stained leucocytes in milk freshly taken (left) and the same sample held refrigerated 24 hr (right).

ing more than 20% oxygen, the group producing less than 20% was found to account completely for the mean day-to-day variation. Means for those in the above 20% group were identical for days 0 and 2 or 0 and 3. Since control programs use values greater than 20%, the variations observed among the higher quality samples should present no problem. These results are in agreement with the relatively consistent results for catalase determinations presented by Tucker and Paape *(6)* when tests were made of milk highly active to the catalase test.

Nageswararao, Blobel and Derbyshire *(3)* believed that "below 20% $0_2$ production, the concentration of substrate was relatively too high, causing rapid inactivation of the enzyme." It is uncertain as to how this relationship could have influenced our experiments, since the same conditions should have existed each day. It is possible that the degeneration of leucocytes which takes place at a high rate after 2-3 days storage could be a factor in the high activity of the samples on those days. This would theoretically increase the enzyme concentration available immediately upon addition of the peroxide to the milk, thus offsetting to some extent the inactivating of the substrate.

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## NEW FASPEEL REDUCES LYE PEELING TIME

A new peeling additive, Faspeel, works wonders eight ways in lye peeling of fruits and vegetables, according to the manufacturer. All that is necessary is to add a very small quantity of Faspeel to the lye peeling solution. Results: (1) Reduced peeling time; (2) More thorough peeling; (3) Rapid penetrating action; (4) Lower peel loss; (5) Excellent rinsing; (6) Lower caustic concentrations; (7) Lower peeling temperatures; (8) Better end-results.

The manufacturer states that in a series of tests, very small quantities of Faspeel added to lye solutions reduced peeling time from 40% to over 60% on tomatoes, apples, and pears. Comparable results can be expected for beets, chili peppers, mangle peppers, eggplant, potatoes, onions, apricots, grapefruit membrane, peaches—any vegetable or fruit that can be lye-peeled. Faspeel is manufactured by J. B. Ford Division, Wyandotte Chemicals Co., Wyandotte, Mich.

## RESIDUAL CHLORINE CONTROL SYSTEM

A control system for continuous measurement of free residual chlorine has been introduced by the Foxboro Co., Foxboro, Mass. Although it is used chiefly in municipal potable-water treatment plants, several applications are found in food processing plants, refineries, chemical plants and other industries where cooling water is chlorinated.

Primary function of the system is to control chlorine addition by regulating a chlorine feeder. It consists of a Model D. Amperometric Cell for measurement and instrumentation for readout and/or control. The cell, a simple device requiring no external energy source or reagent pumps, is designed for field or surface mounting. It continuously monitors the free chlorine level of the process fluid and transmits a proportional signal to an electronic recording-controlling device. The Foxboro analyzer can be used to measure free chlorine as low as 0-1 parts per million or as high as 0-50 parts per million, full scale.