

FACTORS RELATED TO THE ESTIMATION OF CATALASE ACTIVITY IN MILK¹

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SUMMARY

A definite relationship between the somatic cell count of milk and catalase activity was observed when the oxygen liberated was measured accurately with a Warburg apparatus. The somatic cell count could be predicted from the equation: $\log Y = 2.79 + (1.35 \log X)$, where Y = the somatic cell count in thousands, and X = the microliters of oxygen evolved from 0.5 ml of 1% hydrogen peroxide per milliliter of milk in 40 min at 30 C. The correlation coefficient for this regression is 0.90, and is highly significant.

Catalase activity of milk was maximal over the pH range of 5.64 to 10.74 and at 18 C. Activity was close to the maximum at 15 and 22 C and only slightly less at 10 and 25 C. Milk which contained 590 catalase-producing *Staphylococcus aureus* organisms per milliliter liberated very little oxygen; such numbers would not influence conclusions from the catalase test.

The catalase test was recognized as a method for distinguishing between normal and abnormal milk in 1906 (10). It was reported by some investigators (5, 6) to be at least as sensitive as other available tests, but was not recommended for routine use because of the time and equipment required (6). Other investigators (4) reported a poor correlation between leucocyte counts and catalase activity.

Interest in the catalase test was renewed when Spencer and Simon (9) developed a simple method for measuring the oxygen liberated by catalase. Corbett (3) reported results of catalase tests conducted on bulk milk shipments by the Chicago Board of Health modification of the Spencer and Simon test. He noted a good correlation between the catalase test and the California mastitis test.

This investigation was conducted to determine the relationship between numbers of somatic cells in milk and catalase activity, and to study some of the factors (pH, temperature, and catalase-producing bacteria) that might influence this relationship.

EXPERIMENTAL PROCEDURES

Catalase activity.

A Warburg apparatus was used to measure the oxygen liberated. The procedure was as follows: 1.50 ml of milk was placed in the reaction flask. If the somatic cell count was high, the volume of milk was reduced and the difference made up with distilled water to maintain constant volume. Hydrogen peroxide (0.5 ml of a 1% solution) was placed in the side arm of the flask, and 0.20 ml of 20% potassium hydroxide (absorbed on a 1 x 3 cm strip of filter paper) was placed in the center well. After allowing the solution to equilibrate, the hydrogen peroxide was dumped into the milk. The manometer was zeroed and mechanical shaking started (100 strokes per min). The gas evolved was measured at 10-min intervals for 40 min. One flask containing distilled water was used as a thermobarometer to correct for changes in atmospheric pressure or variations in temperature. Results were recorded as the microliters of oxygen liberated from the hydrogen peroxide per milliliter of sample in 40 min at 30 C.

Milk samples.

The milk samples were less than 24 hr old and were obtained from individual cows.

Somatic cell counts.

Numbers of somatic cells were determined by the direct microscopic method (1). Milk smears were stained with Levowitz-Weber stain.

Determination of pH.

A Beckman Laboratory Model G pH meter was used for all pH measurements.

RESULTS AND DISCUSSION

Relation of catalase activity of milk to somatic cell count.

Forty-two samples of raw milk from individual cows were tested in duplicate for numbers of somatic cells and catalase activity. The samples had somatic cell counts ranging from 45,000 to 4,550,000 per ml. The data obtained are shown graphically in Figure 1. The correlation coefficient calculated from the data was 0.90 and the regression calculated by the least squares method was $\log Y = 2.79 + (1.35 \log X)$; where Y = the somatic cell count in thousands, and X = the microliters of oxygen liberated from 0.50 ml of 1% hydrogen peroxide per milliliter of milk.

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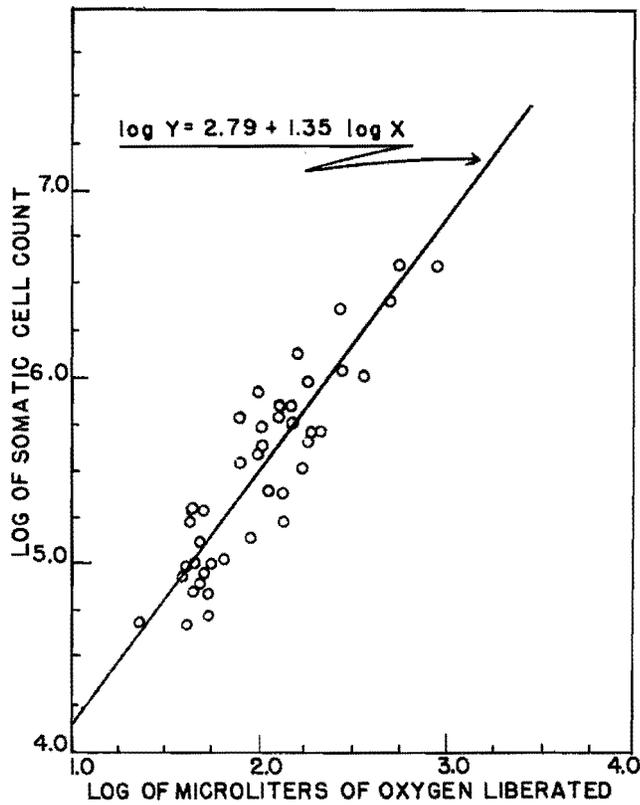


Figure 1. Relationship of the catalase activity of milk to the somatic cell count.

The data indicate that if the oxygen liberated in the catalase test is measured accurately, there is a very significant correlation between numbers of somatic cells and catalase activity.

Effect of pH on catalase activity.

The volume of oxygen liberated by 0.10 ml of raw milk in 30 min at 30 C from 0.50 ml of 1% hydrogen peroxide was determined at several pH values. The milk selected for this experiment was obtained from an animal known to have mastitis. Ellis' universal buffer, adjusted to the desired pH value by adding 0.10 N HCl or 0.10 N NaOH, was used to prepare a milk-buffer mixture (0.10 ml of milk + 1.4 ml of buffer). Catalase activity was determined over the pH range of 3.03 to 11.46.

Data plotted in Figure 2 indicate that catalase activity is maximal over the pH range of 5.64 to 10.74. The decrease in activity was less at pH values below 5.64 than at values above 10.74. No activity was evident at pH 11.46.

For comparison, a sample of blood taken aseptically from a cow was used to determine the effect of pH on catalase activity. The blood was defibrinated and diluted 1:1,000 with 0.85% saline. The data obtained was practically identical to that obtained with milk.

The results reported here, on catalase activity at different pH values, vary somewhat from the results

obtained with other catalase preparations. Chance (2) reported that catalase activity was constant over the pH range of 4.0 to 8.5; above 8.5 activity decreased slowly and fell to about 20% at pH 11.4. Lovrien (7) noted that catalase activity decreased rapidly outside of the range of 3.8 to 10.0.

Variations in the pH of milk should have little effect on the catalase test for the detection of abnormal milk. Mastitis milk generally has a higher pH than normal milk but it never reaches the pH which interferes with catalase activity. Also, appreciable growth of lactic acid-producing bacteria in milk would be necessary to lower the pH value to the point that the enzyme would be inhibited.

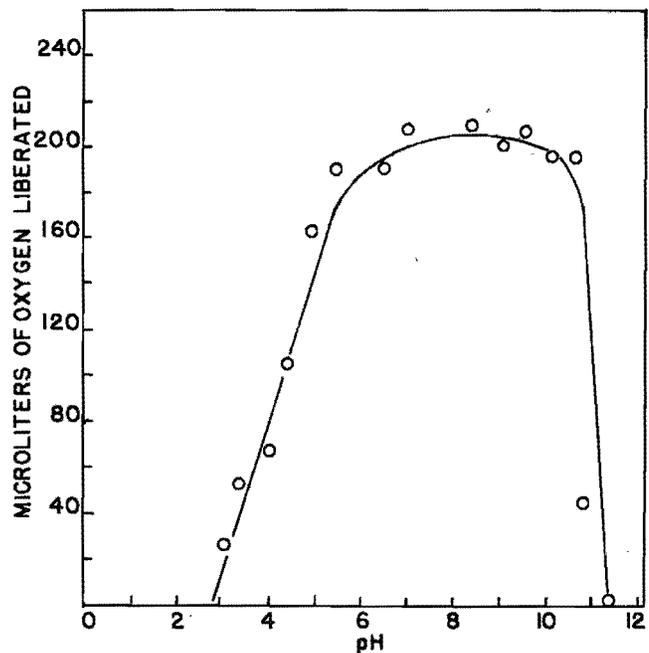


Figure 2. Influence of pH on the catalase activity of milk.

Effect of temperature on catalase activity.

Preliminary trials indicated that the optimum temperature for catalase activity in milk was within the range of 15 to 20 C. To establish the optimum temperature more closely, additional trials were conducted with a sample of milk containing 5,500,000 somatic cells per milliliter. A 0.1-ml portion of this sample was mixed with 1.40 ml of tris (hydroxy methyl) amino methane buffer adjusted to pH 7.2 with 0.1 N HCl.

Figure 3 shows that the optimum temperature for milk catalase activity is 18 C. However, values obtained at 15 and 22 C were very close to the optimum and those obtained at 10 and 25 C were almost identical and only slightly less than the optimum. The catalase activity of defibrinated cow blood was also maximal at 18 C. Morgulis et al. (8) noted

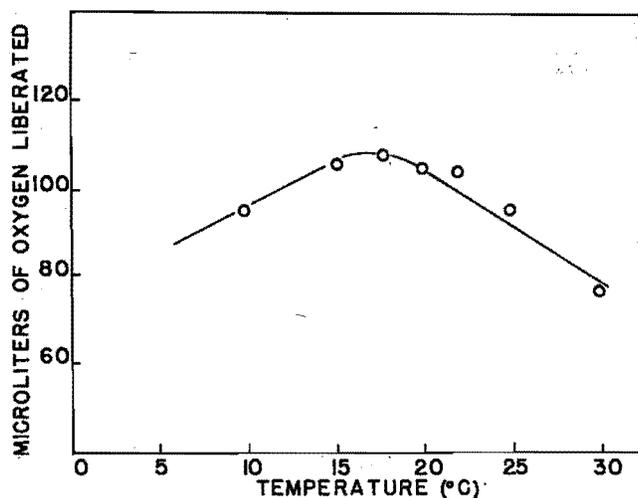


Figure 3. Influence of temperature on the catalase activity of milk.

TABLE I. CATALASE ACTIVITY OF A PURE CULTURE OF *Staphylococcus aureus*.

Incubation time and temperature	Plate count per ml	Microliters of oxygen liberated
Trial 1		
Not incubated	8	5.50
24 hr at 5 C	8	3.70
48 hr at 5 C	6	3.02
48 hr at 5 C + 3 hr at 37 C	10	1.57
Trial 2		
Not incubated	380	2.16
24 hr at 5 C	380	9.36
48 hr. at 5 C	350	2.20
48 hr at 5 C + 3 hr at 37 C	590	6.72

that catalase activity was greatest between 0 and 10 C. Loss of activity was much smaller between 10 and 20 C than between 20 and 30 C. Great loss of activity occurred between 30 and 40 C. Holding catalase for 1 hr at temperatures up to 40 C caused no inactivation of enzyme.

The temperature studies with milk and bovine blood catalase indicate that the catalase test should be conducted within the range of 10 to 25 C. Temperatures above 30 C should not be used even though recommended in some of the earlier published procedures.

Catalase activity of Staphylococcus aureus.

The general opinion is that the catalase detected in milk originates from the cow and is associated with the leucocytes. However, *S. aureus* is a common cause of mastitis and it has a catalase enzyme. Ex-

periments were conducted to determine whether catalase from *S. aureus* might interfere with the relationship of numbers of somatic cells to catalase activity.

A strain of *S. aureus*, isolated from mastitis milk, was added to sterile milk to obtain numbers comparable to those that might be found in raw mastitis milk. The inoculated milk was held at 5 C for 24 hr and for 48 hr, and at 37 C for 3 hr. Plate counts and catalase activity tests were conducted on the samples. Two trials were conducted; in one trial the initial count of *S. aureus* organisms was 8 per milliliter of milk, and in the other trial 380 per milliliter were present.

Data presented in Table I indicate that the strain of *S. aureus* used did not grow in milk at 5 C and very little growth occurred during 3 hr at 37 C. Consequently, little oxygen was liberated. If excessively large numbers of this organism were present they might have an influence on the catalase test. However, most samples of mastitis milk do not contain large numbers of the causative organism and growth is restricted by the holding temperature.

REFERENCES

- American Public Health Association, Inc. 1960. Standard Methods for the Examination of Dairy Products. 11th ed. 1790 Broadway, New York, N. Y.
- Chance, B. 1952. Effect of pH upon the Reaction Kinetics of the Enzyme-Substrate Compounds of Catalase. *J. Biol. Chem.* 194:471.
- Corbett, W. J. 1963. Catalase Test for the Detection of Abnormal Milk. *Milk Ind. Foundation Conv. Proc., Lab. Section*, p. 15.
- Davis, J. G., Newland, L. G., and Knuckey, P. B. 1943. The Resazurin and Rapid Resazurin Tests for Milk - Their Relationship to Other Bacteriological Tests and to Mastitis. *Dairy Inds.* 8:23.
- Gratz, O., and Naray, A. 1912. Comparative Investigations in Regard to the Utility of the Catalase Reductase, and Leucocyte Tests for Detecting Mastitis Milk. *Milchwirtsch. Zentr.* 41:225.
- Hucker, G. J., Trudell, F., and Jennings, W. S. 1932. Mastitis I. The Incidence and Detection of Sub-clinical *Streptococcus* Mastitis. *N. Y. State Agr. Expt. Sta. Bull.* 199.
- Lovrien, R. E. 1959. Physical Chemistry of Catalase. Ph. D. Thesis. Ia. State Univ. Dissertation Abstr. 19:1580.
- Morgulis, S., Berber, M., and Rabkin, I. 1926. Studies on the Effect of Temperature on the Catalase Activity. I. Effect of Different Hydrogen Peroxide Concentrations. *J. Biol. Chem.* 68:521.
- Spencer, G. R., and Simon, J. 1960. The Catalase, California, and Cell Count Tests for Detecting Abnormalities in Milk. *Am. J. Vet. Research* 21:578.
- Trommsdorff, R. 1906. *Berliner tierarztl. Wochen.* 22:282. (Cited by Hucker, G. J. in *The Laboratory Detection of Bovine Mastitis*. *N. Y. State Agr. Expt. Sta. Bull.* 626, 1933).