# THE VALUE OF THE METHYLENE BLUE DYE REDUCTION TEST IN STUDYING NATURAL INHIBITORY PROPERTIES OF MILK

Although Leber (1950) introduced a resazurin test for starter activity, it was criticized by both Golding (1953) and Johns (1953) because milk which contained inhibitory substances such as penicillin gave normal dye reduction times even though titration results were low. Golding also stated that methylene blue was unsuitable for such work.

In spite of these criticisms it was considered that a modified methylene blue test with starter added might prove useful in the routine testing for naturally occurring inhibitory properties of milk, as such milk might well cause lengthened methylene blue reduction times by the starter. It was therefore decided to examine some of the starters which were inhibited in pasteurized milk (activity test) even though they gave normal acidities when used in the vitality test (Gillies 1957).

Both pasteurized and sterilized milks were dispensed in 10 ml quantities and 1 ml of a standard milk testing solution of methylene blue added to each tube. Both types of milk were inoculated with 1 ml of a 1/10 dilution of a 24 hr freshly clotted culture. Methylene blue tests were then carried out at 37°C. The tubes were examined every quarter-hour for reduction of the dye. Results were expressed in methylene blue reduction times. Activity tests were also set up in parallel according to the method of Anderson and Meanwell (1942).

As it was desired to carry out titrations every hour, in each case 2 ml of starter were added to 200 ml of milk. Acidity determinations were performed by first removing 10 ml samples aseptically and then titrating.

The starters used were all strains of *Streptococcus cremoris* and included strains which behaved normally in pasteurized milk as well as those which were inhibited.

Results of the tests are set out in Table 1.

**Table 1**

COMPARISON OF ACIDITY PRODUCTION AND DYE REDUCTION BY "SUSCEPTIBLE" AND "RESISTANT" STRAINS IN BOTH PASTEURIZED AND STERILIZED MILKS

| Susceptible Strain—R6 | | | | | Resistant Strain—C13 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Acidity Produced (%) | | | Methylene Blue Reduction Time (hr) | | Acidity Produced (%) | | | Methylene Blue Reduction Time (hr) | |
| Hours | Pasteurized Milk | Sterilized Milk | Pasteurized Milk | Sterilized Milk | Hours | Pasteurized Milk | Sterilized Milk | Pasteurized Milk | Sterilized Milk |
| 0 | ·16 | ·17 | | | 0 | ·16 | ·17 | | |
| 3 | ·18 | ·20 | | | 3 | ·18 | ·19 | | |
| 4 | ·20 | ·27 | 1¾ | 1¼ | 4 | ·21 | ·24 | 1¾ | 1½ |
| 5 | ·22 | ·38 | | | 5 | ·32 | ·30 | | |
| 6 | ·24 | ·50 | | | 6 | ·46 | ·40 | | |

It is apparent from these results that there is no correlation whatever between acidity developed by any of the cultures in pasteurized or sterilized milks and the methylene blue reduction times. Susceptible and resistant strains gave the same dye reduction time in pasteurized milks, yet the acidity developed by the susceptible strain was approximately half that developed by the resistant strain.

However, when it is considered that reduction of the methylene blue occurred before any differences in acidity production were apparent, such a test is no real indication of any inhibitory properties in the milk. Accordingly, decreased inocula were used, so that the methylene blue times in both types of milk were increased and it was therefore possible to obtain a true comparison between the methylene blue reduction and the acidity developed. Such a series of results is expressed in Table 2.

**Table 2**

COMPARISON OF METHYLENE BLUE REDUCTION TIMES OF "SUSCEPTIBLE" AND "RESISTANT" STRAINS IN PASTEURIZED AND STERILIZED MILK USING DIFFERENT INOCULA. (ACTIVITY TEST RESULTS ARE ALSO INCLUDED)

| | Methylene Blue Reduction Times in Hours | | | |
|---|---|---|---|---|
| | Susceptible Strains | | Resistant Strains | |
| | HP | R6 | C2 | C3 |
| *Sterilized milk—* | | | | |
| 1% inoculum .. .. | ¾ | ¾ | 1 | 1½ |
| ·2% inoculum .. .. | 1¾ | 2¼ | 1½ | 1½ |
| *Pasteurized milk—* | | | | |
| 1% inoculum .. .. | 4½ | 6 | 2¼ | 2½ |
| ·2% inoculum .. .. | >7½ | >7½ | 4 | 4 |
| | Percentage acidity developed using 1% inoculum | | | |
| Sterilized milk .. .. | ·38 | ·43 | ·42 | ·46 |
| Pasteurized milk .. .. | ·22 | ·22 | ·36 | ·40 |

It is evident from Table 2 that it is possible, by the use of decreased inocula, to obtain a methylene blue reduction time which correlates with the acidity developed by the susceptible strains in pasteurized milks. It is also apparent that the reduction times for all strains (susceptible and resistant) are longer in pasteurized milks than in sterilized milks, evidently due to changes in the reducing systems by sterilization. In this point the two tests differ, as resistant strains are able to produce comparable quantities of acid in both pasteurized and sterilized milks.

Although it has been possible to obtain methylene blue reduction times which reflect the presence of naturally occurring inhibitory properties in the milk, the time taken to complete both activity and methylene blue reduction tests is comparable. The principal advantage in the methylene blue reduction test lies in the fact that it is more easily adapted to field use than the activity test.

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