UPON THE REDUCTION OF METHYLENE BLUE BY COW'S MILK.

By E. P. CATHCART, M.D.

(*From the Bacteriological Department, Lister Institute.*)

H. Smidt in a recent note on the reduction of methylene blue by means of milk[1] confirmed the results of Schardinger that a mixture of formaldehyde and methylene blue is decolorised by the action of milk. This reaction depends on the presence of a ferment—a catalase—in the milk, and, as this ferment is readily destroyed by heat, serves as a very simple method of determining whether a given sample of milk is "raw" or not. By way of conclusion Smidt stated that he thought this test might be of still further utility in that it might be possible to estimate the degree of bacterial contamination of the milk by its means. If such a comparatively simple test could be employed, in an easy and effective fashion, it would be of immense service, as, owing to the rapidity with which it can be carried out, the milk could be examined before distribution.

As the possibilities of this test seemed to be large I carried out a few experiments. In the first place it was of some importance to know whether the time required for reduction was independent of temperature, within reasonable limits.

The experiments were performed in a water-bath constant to within 1 degree when used with care, made of glass, so that the test-tubes, containing the milk and methylene blue, could be observed without being disturbed. The quantity of milk used was 10 c.c., pipetted into test-tubes of as near equal bore as could be obtained, and to this milk was added 0·5 c.c. of Smidt's methylene blue solution[2]. The blue and the

[1] *Hygien. Rundsch.* No. 23, 1903.

[2] Methylene blue solution. Saturated meth. blue alcoholic (absolute) sol. 5 c.c.
Formaldehyde sol. 5 c.c.
Aq. dist. 190 c.c.

https://doi.org/10.1017/S0022172400002953 Published online by Cambridge University Press

milk were mixed by shaking and then placed in a circular test-tube rack which was immersed in the water-bath. It was found that the variation in time required for reduction, between the temperature of 40 and 50 degrees was very considerable. This demonstrates in the first place the fact that, if the temperature were not very closely controlled, great mistakes as to the reducing power of the milk, and so as to its bacterial content, might easily be made. A rise of 10° caused a diminution of practically 50 per cent. in the time of reduction.

TABLE I.

Milk (fresh), 10 c.c. 0·5 meth. blue sol.

Temp. of bath	Time required for reduction
40°	(*a*) 15 mins. (*b*) 15 mins. 30 secs.
45°	(*a*) 10 mins. 40 secs. (*b*) 11 mins.
50°	(*a*) 7 mins. 45 secs. (*b*) 8 mins.

Thus it will be seen that to get anything like reliable and constant figures a very definite temperature must be maintained.

Another point which was of interest was to see whether the temperature of the milk itself played any part. This was important as in some instances the milk might be brought for examination in an ice box and a sample taken for immediate testing. Here again another possible source of error was detected. It must be admitted, however, that the difference was not very marked.

TABLE II.

Water-bath at 50°.

(*a*) 10 c.c. milk at room temp. (18° C.) had 0·5 c.c. meth. blue added and then placed in the bath. Time for reduct. 9 mins. 30 secs.

(*b*) 10 c.c. of same milk, but previously in bath at 50° for 7 mins., had 0·5 c.c. meth. blue added, then returned to bath. Time for reduct. 8 mins. 45 secs.

As the catalase is found almost completely in the cream, as is readily proved by centrifugalising the milk, it was interesting to see if allowing the milk to stand for a few hours and then taking two samples, one from the top layer the other from the bottom, would alter the times obtained. Milk was accordingly left absolutely still for some three hours and a sample taken from the top layer by means of a pipette and another from the bottom of the vessel. Here again a result was obtained in favour of the multiplication of errors.

TABLE III.

(*a*) Milk from bottom reduced in 8 mins. 45 secs.
(*b*) ,, top ,, ,, 6 mins. 30 secs.

Some experiments were also carried out as regards the effect of previously heating the milk. The results showed that the catalase is very sensitive to the action of heat. According to my results the point of destruction lies somewhere between 65 and 70 degrees. This figure is a few degrees lower than that given by Smidt.

TABLE IV.

Milk and meth. blue as usual. Bath 50°.

Temp.	Time of exposure	Time required for reduction
60°	10 mins.	10 mins. 45 secs.
	20 mins.	12 mins. 50 secs.
	30 mins.	13 mins. 15 secs.
65°	10 mins.	8 mins. 15 secs.
	20 mins.	16 mins.
	30 mins.	Slight in 30 mins.
70°	10 mins.	30 mins. no reduction.
	20 mins.	,, ,, ,,
	30 mins.	,, ,, ,,

Control (unheated milk) 7 mins. 50 secs.

Other experiments, roughly quantitative in nature, were performed in order to see whether or not the catalase had been injuriously affected by heating at 60°. Here again the milk was exposed for different periods at the given temperature. In this case known volumes of the methylene blue solution were added until the milk ceased to decolorise it. As will be seen from the following table, the catalase had been affected most by the 30 minutes exposure. There is even a slight difference between that exposed for 10 minutes and the control. In this experiment the milk which was used was one day old but had been kept in the ice box overnight.

TABLE V.

Amount of meth. blue	Milk control	Milk heated to 60° for 10 mins.	20 mins.	30 mins.
0·5 c.c.	2 mins. 15 secs.	2 mins. 45 secs.	3 mins. 0 secs.	3 mins. 20 secs.
+0·5 c.c.	3 ,, 25 ,,	2 ,, 30 ,,	3 ,, 30 ,,	6 ,, 10 ,,
+0·5 c.c.	5 ,, 0 ,,	6 ,, 0 ,,	9 ,, 5 ,,	Slight reduct. in 30 mins.
+0·5 c.c.	Slight reduct. in 30 mins.	No reduct. in 30 mins.	No reduct. in 30 mins.	

https://doi.org/10.1017/S0022172400002953 Published online by Cambridge University Press

This experiment further serves to demonstrate the fact that old milk reduces methylene blue more rapidly than fresh. This of course is presumably due to the increased bacterial content.

As so many factors, which of themselves might not be looked on as particularly weighty, seem to affect the easy carrying out of this test it was considered of importance to see what the result of adding varying amounts of an organism like *B. coli* might be. As will be seen from the table it was only on adoption of the quantitative method that one got any result, and at the best the figures obtained are not very striking.

TABLE VI.

Milk 10 c.c. + meth. blue + broth cult. of *B. coli*. Bath at 50°.

Amount of meth. blue	Amount of *B. coli* cult. 0·2 c.c.	0·5 c.c.	1 c.c.
0·5 c.c.	9 mins. 50 secs.	9 mins. 45 secs.	9 mins. 30 secs.
0·5 c.c.	1 min. 45 secs.	1 min. 45 secs.	1 min. 25 secs.
1·0 c.c.	No reduct. in 30 mins.	Slight reduct. in 30 mins.	About half reduced in 30 mins.

Another source of error which I should imagine would prove fatal to the quantitative use of this test as a means of finding the bacterial contamination of milk is that the reducing action of organisms varies to a marked degree. In a paper published by Prof. Hahn and myself[1] we showed this variation very clearly. The following table, in order to demonstrate this point, is extracted from the paper in question.

TABLE VII.

Reduction of 0·5 c.c. 1/10 % meth. blue sol. by 10 c.c. bouillon culture.

Culture and age	Reduction time Mins.	Secs.
Bac. coli (18 hours)	5	10
Bac. typhi (18 hours)	20	—
Bac. prodigiosus (2 days)	18	30
Staphylococcus aureus (2 days)	6	—
Bac. lact. aerogenes (2 days)	7	—
Bac. subtilis (2 days)	Only slight reduct. in 1 hour.	
Bac. diphtheriae (2 days)	—	

Conclusions:—The methylene blue formaline test serves most excellently to differentiate between boiled (or heated) and "raw" fresh milk. As a means of estimating the bacterial contamination of the milk it seems to me to be too delicate for ordinary rough and ready use. It might possibly be of value in a large laboratory where the various sources of error could be readily eliminated.

[1] *Arch. f. Hygiene*, vol. XLIV. p. 296.

https://doi.org/10.1017/S0022172400002953 Published online by Cambridge University Press