THE INFLUENCE OF DDT WETTABLE POWDER ON THE METHYLENE BLUE REDUCTION TEST IN MILK

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DDT has been detected in the milk of cows fed forage crops exposed to the insecticide. DDT, in the form of a wettable powder, was added to raw milk to determine its effect on the methylene blue reduction test. The results indicate that the presence of appreciable quantities of DDT wettable powder materially interfere with the accuracy of the test. Care should be exercised in interpreting reduction tests of milk containing this insecticide.

The methylene blue reduction test has been widely accepted in the dairy industry as a qualitative method for checking the sanitary quality of raw milk. The rapidity of the reduction time of the dye in milk may be influenced by several factors, particularly the kind of organisms in the milk. Recently, the use of various insecticides on forage crops has introduced a relatively new factor. If DDT is present in the milk in varying quantities, what will be the effect of this insecticide on the accuracy of the methylene blue test? Different concentrations of a DDT wettable powder were added to raw milk samples produced under a variety of sanitary conditions. No forage crops containing DDT were available at the time of this investigation.

Review of Literature

Many inherent and environmental factors may influence the relationship of dye reduction and the numbers and/or kinds of organisms present in the milk. Davis and Lines[2] have noted that the concentration of dye plays an important role in the reduction time. The temperature of incubation of the dye-milk mixture as reported by Hastings *et al.*[4] showed a wide variation in the time of reduction of the methylene blue. The pre-test holding-temperature as noted by Frayer[3] was another factor and was confirmed by Wilson.[11] The work of Aikins and Fay,[1] Frayer[3] and Jackson[7] indicated that light may be an important factor in the reduction time, and they suggested that the tubes should be protected from light rays.

Schecter *et al.*[8,9] and Howell *et al.*[6] reported that DDT could be detected in milk in concentrations of 25 ppm and higher depending on the cow's intake of DDT present as residue on the forage crops the animal consumed.

These observations prompted us to investigate the possibility that DDT also might interfere with the accuracy of the methylene blue reduction test in milk.

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Materials

Since milk was not available from cows fed DDT forage crops, *in vitro* tests were performed using a 50 percent DDT wettable powder insecticide prepared by E. I. du Pont De Nemours and Co. and distributed under the trade name of "Deenate 50W".

The formulation of this water-dispersible powder is as follows:

95% by weight .... concentrate A
0.5 to 1% .... wetting agent
1 to 3% .... dispersing agent
1 to 3.5% .... inert diluent
(du Pont dry concentrate A contains 53 percent technical DDT by weight)

One gram of Deenate dispersed in 99 ml of distilled water was used as a stock solution from which higher dilutions were made. A fresh solution was prepared monthly.

The milk samples were kept around 27°C for not more than 2 hours before being used.

Procedure

Ten-ml portions of (a) fresh poor quality, (b) fresh high quality and (3) week-old high quality raw milk containing various concentrations of Deenate (10, 100, 1,000 and 0 ppm, the controls) were added to test tubes holding 1 ml of a methylene blue thiocyanate solution. (The dye solution was prepared by dissolving one methylene blue thiocyanate tablet, approved by the Biological Stain Commission, in 200 ml of distilled water. Fresh dye solution were prepared weekly and stored in an amber bottle.) Each tube was inverted three times to insure uniform cream and dye distribution before it was placed in a 37°C water bath.

TABLE 1 — THE INFLUENCE OF A DDT WETTABLE POWDER ON THE TIME REQUIRED FOR THE REDUCTION OF METHYLENE BLUE IN RAW MILK.

RAW MILK		DEENATE		REDUCTION TIME (HR.)							
Quality	Age	ppm		0.5	1	2	3	4	5	6	7
Low	Fresh	0	(control)	–	–	–	–	–	+	+	+
		10		p–	p–	p–	p–	p–	p+	p+	p+
		100		p–	p–	p–	p–	p–	p+	p+	p+
		1000		p–	p+	p+	p+	p+	p+	p+	p+
High	Week-old	0	(control)	+	+	+	+	+	+	+	+
		10		+	+	+	+	+	+	+	+
		100		+	+	+	+	+	+	+	+
		1000		+	+	+	+	+	+	+	+
High	Fresh	0	(control)	–	–	–	–	–	–	–	+
		10		p–	p–	p–	p–	p–	p–	p–	p+
		100		p–	p–	p–	p–	p–	p–	p–	p+
		1000		p–	p+	p+	p+	p+	p+	p+	p+

– no change in color of milk
\+ dye reduction
p– dye precipitation, milk decolorized slightly
p+ dye precipitation, milk decolorized completely

The colors of the samples were checked after 30 min incubation. Readings were then made at hourly intervals following the initial reading. The decolorized samples were removed from the water bath and the remaining tubes were inverted once. Decolorization was considered complete when 4/5 of the tube contents were white.

The effects of various concentrations of a DDT wettable powder on the time required for the decolorization of a methylene blue thiocyanate solution when added to low quality milk, milk of high quality but held for 7 days at 15°F, and milk of high quality but not stored, are shown in table 1.

RESULTS

The methylene blue in test (10, 100, 1000 ppm Deenate) and control samples (0 ppm Deenate) of week-old high quality raw milk was reduced within 30 min. The dye in the control samples in both low and high quality fresh raw milk was reduced after 5 and 7 hrs., respectively.

Blue colored particles precipitated in both fresh low and high quality raw milk containing Deenate as soon as they were inverted. The amount of precipitated material varied as did the Deenate concentration; small precipitates were found in samples containing 10 ppm Deenate and correspondingly larger precipitates occurred in samples containing 100 and 1,000 ppm. A corresponding reduction accompanied the dye precipitation; samples containing 1,000 ppm Deenate were completely decolorized within 60 min. Lower concentrations of Deenate did not appreciably affect the reduction time in the milk.

The addition of a small amount of Deenate, 10 ppm, to an aqueous methylene blue solution caused the dye to precipitate. This phenomenon failed to occur when pure DDT crystals were added to this solution.

DISCUSSION

The results seem to indicate that the presence of an appreciable quantity of a DDT wettable powder in raw milk materially interferes with the accuracy of the Methylene Blue Reduction Test. Deenate has been found to precipitate the dye in fresh raw milk before the natural reducing system of the milk (Thornton and Hastings,[10] or the reducing substances formed by the microorganisms in the milk (Hobbs,[5]) could affect a reduction of the methylene blue. Furthermore, the "active agent" of the insecticide, the DDT, did not cause the precipitation; the so-called "nnert constituents" were the responsible agents.

If more were known about the specific nature of these "inert constituents", it might be possible to postulate a mechanism to explain this phenomenon. There probably are several contributing factors, as many common wetting and and dispersing agents failed to precipitate the dye when used individually.

The significance of these observations is in the interpretation given the Methylene Blue Reduction Test run on fresh high grade milk containing significant amounts of Deenate. Milk of this sort might easily be mistaken for old or low grade milk. To obviate this danger, rapidly decolorized milk samples should be inspected for the presence of precipitates before final interpretations are made.

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TABLE 1 — PRELIMINARY COMPARATIVE DATA

Sample	Percent of tubes showing gas develp. Brilliant-green bile				Number of colonies on membrane filters Endo-agar			
Ml of milk	1	10	50	100	1	10	50	100
Pasteurized milk	0	0	60	100	0.0	0.0	2.1	5.7
Pasteurized milk	40	100	100	100	2.0	8.1	27.0	TNTC
Pasteurized milk	0	0	0	40	0.0	0.0	0.0	1.8
Pasteurized milk	0	0	40	80	0.0	2.0	6.3	25.0
Certified milk	0	0	0	20	0.0	0.0	0.0	2.2
Certified milk	0	0	0	0	0.0	2.2	2.7	3.2
Certified milk	60	40	60	100	7.2	9.7	TNTC	TNTC
Certified milk	0	0	20	80	0.0	1.1	7.4	11.2

zero coliform, it was established to the satisfaction of the author that coliform content secured on milk samples tested was from the milk source and not a result of technique contamination.

SUMMARY

Preliminary work on the use of a membrane-filter method for determination of coliforms organisms is presented. This method permits more rapid counting of coliform colonies present and eliminates much of the plating heretofore necessary. The results of the counting can be preserved directly on the membranes for future reference. The use of the centrifuge in the method outlined permitted actual examination of large quantities of milk.

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