



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 qual-

ity 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,<sup>10</sup> or the reducing substances formed by the microorganisms in the milk (Hobbs,<sup>5</sup>) 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 "inert 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 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 develop.				Number of colonies on membrane filters			
	Brilliant-green bile				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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