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Thermal Death Time Curve of *Mycobacterium tuberculosis* var. *bovis* in Artificially Infected Milk[1]

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An accurate estimate of the slope of the thermal death time curve of *Mycobacterium tuberculosis* var. *bovis* in milk has not been reported, and only a few attempts to construct such curves for this organism have been made (North and Park, 1927; Oldenbush *et al.*, 1930). Ball (1943) concluded that none of the thermal resistance data reported in the literature was applicable to the construction of thermal death time curves for *M. tuberculosis* in milk. A possible nonlinearity in the curve at temperatures above 67 C was indicated by Faxholm (1949).

The project reported here was undertaken to study the characteristics of the thermal death time curve of *M. tuberculosis* var. *bovis* in milk.

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EXPERIMENTAL METHODS

Three strains of *M. tuberculosis* var. *bovis* were employed, strain USDA 854, strain ATCC 11756, and strain ATCC 12621. Cultures were carried on slants of Dubos medium (Dubos and Middlebrook, 1947), and cells for the test suspensions were prepared by obtaining a submerged, dispersed growth in Dubos Tween-albumin medium (Dubos and Middlebrook, 1947). Cells were harvested by centrifugation after 8 to 10 days of incubation on a shaker bath at 37 C, washed three times in physiological saline containing 0.05 per cent Tween 80,[3] and resuspended in the same saline-Tween diluent. The extent of cell clumping was controlled by shaking with glass beads and filtration through two sheets of Schleicher[4] 597 filter paper. The degree of cell clumping was determined microscopically. Viable cell counts were performed on Dubos oleic-acid

[3] Polyoxyethylene sorbitan monooleate, General Biochemicals, Inc., Chagrin Falls, Ohio.

Figure 1. Thermal death time curve of *Mycobacterium tuberculosis* var. *bovis* 12621 in milk.
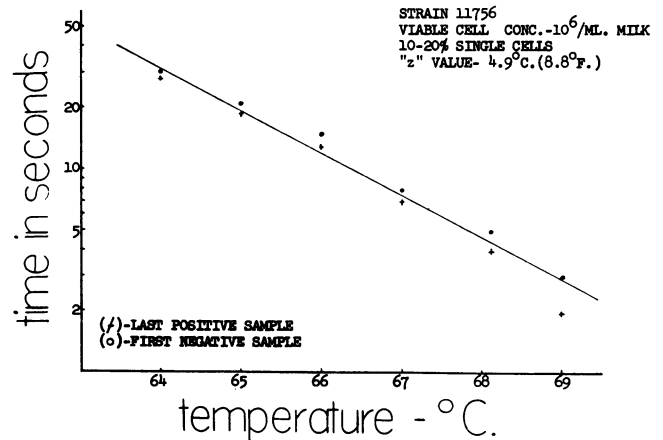

Figure 2. Thermal death time curve of *Mycobacterium tuberculosis* var. *bovis* 11756 in milk.

albumin medium (Dubos and Middlebrook, 1947) according to Fenner et al. (1949). The counts were reported only approximately because of the cell clumping in the suspensions.

The thermal death time equipment was essentially the same as that employed by Kronenwett et al. (1954) and Bachmann (1954). This equipment provided instantaneous heating and efficient mixing of the test suspension in the milk, accurate sampling at short-time intervals, and rapid cooling of the samples. Samples injected into the heating bath were uniformly mixed 2 sec after injection, and experimentally determined cooling curves indicated that negligible lethal heat was applied to the test organisms during the cooling period.

Surviving organisms were recovered in Dubos liquid medium containing 0.0001 per cent malachite green without Tween 80. Incubation of these tubes for 3 to 4 weeks at 37 C was followed by transfer to slants of the same medium as a confirmation step. After 4 weeks of

[4] Carl Schleicher and Schuell Company, Keene, New Hampshire
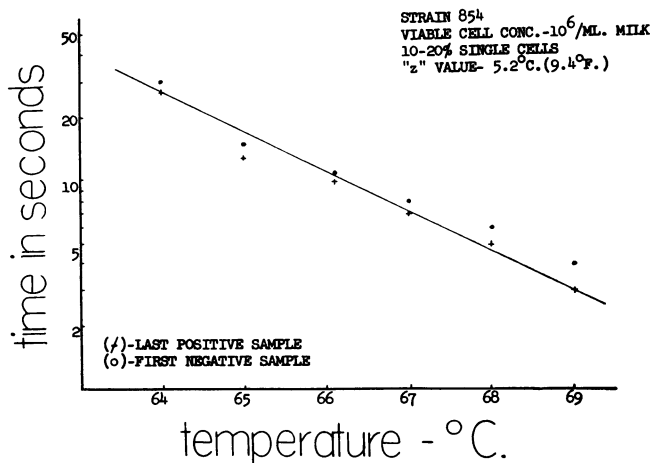

Figure 3. Thermal death time curve of *Mycobacterium tuberculosis* var. *bovis* 854 in milk.
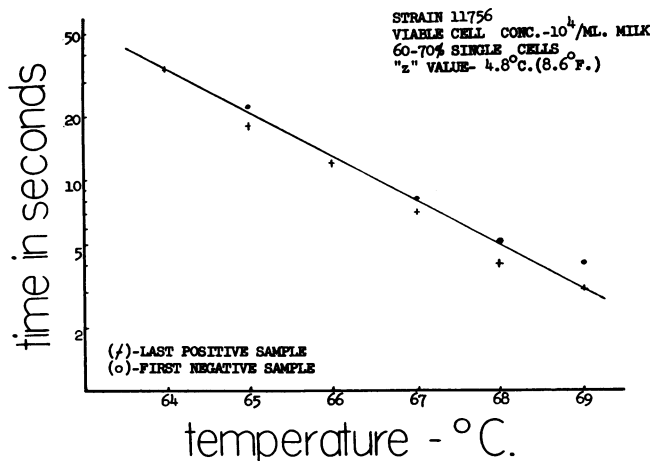

Figure 4. Thermal death time curve of *Mycobacterium tuberculosis* var. *bovis* 11756 in milk.

incubation at 37 C, the slants were examined for the presence of characteristic colonies.

RESULTS AND DISCUSSION

A thermal death time curve was determined for each strain in the temperature range of 64 to 69 C, and the effect of cell clumping upon the z value of *M. tuberculosis* var. *bovis* in milk was investigated. The experimental thermal death time curves are presented in figures 1 through 4, and the results of these studies are summarized in table 1. The z values obtained for the strains employed range from 4.8 C (8.6 F) to 5.2 C (9.4 F), and are considerably lower than the z value of 12.4 F calculated by Ball (1943) from the existing data. All curves obtained were linear in the temperature range of 64 to 69 C. The extent of cell clumping in the test suspension appeared to have no effect upon the z value obtained for strain 11756. Figure 5 depicts the comparison of the thermal death time curve of strain 11756 at the maximum probable concentration to be found in naturally contaminated milk, the curve reported by Ball (1943), and the curve drawn through the present pasteurization standards for milk. These results indicate that the present pasteurization standards provide a margin of safety of approximately 28½ min at 143 F, and approximately 14 sec at 161 F. These margins of safety
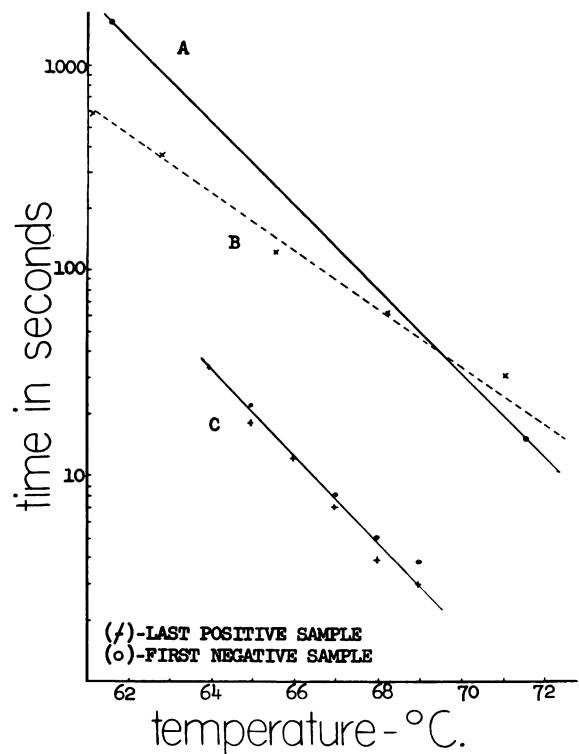

Figure 5. Comparison of thermal resistance curves with pasteurization curve. *A*, curve drawn through present milk pasteurization standards. *B*, thermal death time curve for *Mycobacterium tuberculosis* in milk from Ball (1943), z value = 12.4 F. *C*, experimental thermal death time curve for *M. tuberculosis* var. *bovis* 11756 (conc = 10⁴/ml milk, z value = 8.6 F).

may, in fact, be greater, because the present pasteurization standards represent holding times and temperatures, and ignore any lethal heat which may be applied in the pre-holding and cooling periods.

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TABLE 1

Thermal death time data for three strains of Mycobacterium tuberculosis var. bovis in milk

Strain	Approximate Viable Cell Conc per Ml Milk	Cell Dispersion		Thermal Death Time in Sec	z Value*
		Per cent single cells	Remainder (cells per clump)		
ATCC 12621	10^6	10–20	2–10	64.0 C >38 <41	4.8 C (8.6 F)
				65.0 C >19 <22	
				66.0 C >13 <15	
				67.0 C >8 <9	
				68.0 C >5 <6	
				69.0 C >3 <4	
ATCC 11756	10^6	10–20	2–10	64.0 C >28 <30	4.9 C (8.8 F)
				65.0 C >19 <21	
				66.0 C >13 <15	
				67.0 C >7 <8	
				68.1 C >4 <5	
				69.0 C >2 <3	
USDA 854	10^6	10–20	2–10	64.0 C >27 <30	5.2 C (9.4 F)
				65.0 C >13 <15	
				66.1 C >10 <11	
				67.0 C >7 <8	
				68.0 C >5 <6	
				69.0 C >3 <4	
ATCC 11756	10^4	60–70	2–5	64.0 C >34 —	4.8 C (8.6 F)
				65.0 C >18 <22	
				66.0 C >12 —	
				67.0 C >7 <8	
				68.0 C >5 <6	
				69.0 C >3 <4	

* Determined from the thermal death time curve. Equivalent to the number of degrees of temperature required for the curve to pass through one log cycle.

laboratory work. The authors are indebted to Dr. E. L. Love of the Animal Disease Eradication Branch of the United States Department of Agriculture for supplying one of the cultures, and Dr. C. Olin Ball for reviewing the manuscript.

SUMMARY

The range in z values determined for three strains of Mycobacterium tuberculosis var. bovis in milk was 4.8 C (8.6 F) to 5.2 C (9.4 F). The thermal death time curves of the three strains were found to be linear in the range of 64 to 69 C. The extent of cell clumping in the test suspensions had no effect upon the z values obtained for strain 11756. The results obtained employing a cell concentration comparable to the maximum probable concentration to be found in naturally infected milk indicate that the present pasteurization standards provide a margin of safety of approximately 28½ min at 143 F, and approximately 14 sec at 161 F.

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