APPLICATION OF THE DECIMAL REDUCTION TIME PRINCIPLE TO A STUDY OF THE RESISTANCE OF COLIFORM BACTERIA TO PASTEURIZATION[1]

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Although it has been repeatedly observed that the thermal death time of bacteria is dependent upon the temperature employed (Ballner, 1902; Meyer, 1906; Sattler, 1928), the initial concentration of organisms (Chick, 1910; Ficker, 1898; Madsen and Nyman, 1907; Reichenbach, 1911), the test medium (Brown and Peiser, 1916; Ficker, 1898; etc.), and the physiological age of the culture (Ficker, 1898; Reichenbach, 1911; Schultz and Ritz, 1910; Sherman and Albus, 1923), these factors, especially the influence of the initial concentration of bacteria, have been ignored by a number of workers. When this factor is considered, the thermal death time follows a regular order which is adequately described by applying the equation for the "mono-molecular reaction rate" (Baker and McClung, 1939; Bigelow, 1922; Chick, 1910; Madsen and Nyman, 1907). This has been generally ignored by workers interested in thermal death, and as a result the literature contains a number of conflicting statements in this regard. This has made it difficult to evaluate some of the bactericidal aspects of the pasteurization process. As an aid in quantitative determinations of bacterial death by heat, the authors have proposed the concept of "decimal reduction time" (Katzin and Sandholzer, 1942) which is based upon the application of the mono-molecular reaction rate constant to bacterial death under uniform conditions. The purpose of this study is to illustrate the application of this principle to the problem of the use of coliform bacteria as indicators of the sanitary status of pasteurized milk.

MATERIALS AND METHODS

Cultures

In all, 66 strains of *Escherichia* were employed. These were originally isolated from a variety of sources including human, monkey, bovine and sheep feces, human blood, soil, milk, water, urine and oysters. The initial source of some of the older laboratory strains is uncertain.

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[4] The impetus for this investigation was given by a study of unpublished data collected by Messrs. Frank, Fuchs, Moss *et al.* in the files of the U. S. Public Health Service.

Methods

Ten ml. volumes of peptone-water (Bacto)[5] were seeded with 1 ml. of a 24-hour culture of the test organism grown in the same medium at 37°C. After incubation for 24 hours, portions of the test culture were inoculated into whole milk (equal parts of evaporated milk and sterile water). Aliquots of culture were chosen which would yield approximately one million bacteria per milliliter of milk. Aliquots of inoculated milk were removed for determining the initial plate count on nutrient agar, the remainder being employed for experimental pasteurization.

Quadruplicate tubes, one-half inch in outer diameter, were each apportioned 3 ml. of inoculated milk respectively for each strain tested. Two of these were removed after being heated for 3.0 minutes and the remaining two after 4.5 minutes. Twelve strains were usually heated at one time to insure uniformity of the experimental conditions and to assure comparable data.

Pasteurization was accomplished in a thermostatically controlled bath (constant to 0.1°C.) which brought the fluid in the tubes to a final temperature of 61°C. (141.8°F.) within 1½ minutes. The milk surface was kept two or three centimeters below the surface of the water in the bath, and the tubes were constantly agitated.

At the end of the heating period the tubes were quenched in ice water. A 1:100 dilution of the heated milk was made in 0.85 percent sodium chloride solution, and the number of survivors was determined by the "most probable number" technic, using lactose broth as the test medium. Five tubes at each of 3 decimal dilutions yielded results of sufficient accuracy.

Calculations

The decimal reduction time is defined by

$$\text{DRT} = 2.3/k = (t_2 - t_1)/\log_{10}(C_1/C_2) \tag{1}$$

(see Katzin and Sandholzer, 1942) where k is the monomolecular reaction rate constant, and C_1 and C_2 are the initial and final concentrations of bacteria, respectively, subjected to a constant lethal temperature for $(t_2 - t_1)$ minutes. A sample calculation will illustrate the use of the relationship: milk having an initial concentration of coliform bacteria of 1.7 million cells per milliliter contains 23 cells per ml. after 3.0 minutes heating at 61°C.

$$\log_{10}(C_2/C_1) = \log_{10}(1{,}700{,}000/23) = \log_{10}(73700) = 4.87 \tag{2}$$

$$\text{DRT}_{61°} = 3.0/4.87 = 0.62 \text{ minutes} \tag{3}$$

[5] The formula used was the following:

Peptone (Bacto)	20 g
NaCl	5 g
Water	1000 ml.

Adjust to pH 7.4–7.6. Autoclave at 20 lbs. pressure for 15 minutes.

RESULTS

A sample of the data obtained is shown in table 1. A larger proportion of the strains than is indicated gave zero counts in the shortest heating time tried. In some cases, counts of two organisms per ml. (one tube of fifteen positive) are no doubt indications that some small portion of fluid, such as wets the wall of the test tube through surface tension, did not get heated equally with the rest of the sample. Similar sources of growth account for other cases where small counts are obtained that are out of line ("skips"). Not all minimal counts are to be construed in this fashion, however. Counts indicated as >1600 or >1400 are cases where no tubes showed a negative test for coliforms (one negative tube gives a count of 1600 in the present case; see Hoskins, 1934). If no counts as

TABLE 1

Determination of decimal reduction time of coliform bacteria sample data

CULTURE NUMBER	INITIAL COUNT IN MILLIONS	M.P.N. PER ML. AFTER 3.0 MINS. AT 61°C.		M.P.N. PER ML. AFTER 4.5 MINS. AT 61°C.		MEAN VALUE $\log_{10}(C_1/C_2)$		PROBABLE DRT
		A	B	A	B	3.0 mins.	4.5 mins.	
	per ml.							*minutes*
F-1	.58	11	2	13	0	5.1	5.1	.59
324	2.7	0	2	0	0	>6.2		<.48
47	>8	2	4.5	0	0	>6.4		<.47
249	1.0	>1400	1100	4.5	2		5.5	.82
319	2.8	130	350	2	2	4.1	6.2	.73
244-1	5	13	23	0	0	5.5		.55
234-3	2.7	33	13	0	0	5.1		.59
56L	5	0	0	0	0	>6.7		<.44
M-2	1.9	>1600	>1600	350	920		3.5	1.3
1B	3.1	>1600	>1600	2	2		6.2	.73
3B	4	350	350	2	0	4.1	>6.5	.73
231-1	.58	0	0	0	0	>5.8		<.52
231-2	2.8	2	4	0	0	6.0		.50

low as 1600 were obtained in 4.5 minutes heating, it became necessary to do another experiment employing either lower initial concentrations of bacteria, longer periods of heating, or both.

The advantages of the "most probable number" technique over plate counts lie in the lower threshold sensitivity and greater counting range. The range may be extended to lower thresholds or to higher counts by appropriate use of more tubes. When, as with strains 319 and 3B, the data allow calculation of the DRT from both heating times, the agreement is often remarkably close. Strain F-1 gave very consistent results on repeated testing. In some cases, several smooth and rough variants of the same strain were tested and showed no significant difference of DRT (231-1 and 231-2, for example). It should not be assumed that correspondence to better than 10% always results, but with care in experimental manipulation it will obtain more often than not. A considerable part

of the reproducibility of the results stems from the large change in numbers of the bacteria.

Table 2 is a summary of DRTs for the 66 strains tested. Where necessary, the extreme, median and modal DRT values obtained are shown, to indicate the distribution. As can be seen, they tend to be less than 0.5 minute in the majority of the cases. Only four strains showed a DRT over one minute, and none were found as high as two minutes. The values would have been even lower, if shorter times had been used, and if proper correction could be made for the interval in which the tubes were below the terminal temperature. Special rate experiments on strain F-1 indicated a true DRT nearer 0.3 minute. The inaccuracies were less with high values of DRT. It is uncertain whether the tend-

TABLE 2

The decimal reduction time at 61°C. of coliform bacteria from various sources

SOURCE OF CULTURES	NUMBER OF STRAINS	DRT	MEDIAN DRT	MODAL DRT
		minutes		
Bovine feces	4	0.7 -0.9	0.7	0.7
Human feces	7	<0.3 -0.7	<0.6	<0.6
Monkey feces	2	<0.5 -0.7		
Sheep feces	1	1.3		
Blood (cadaver)	1	0.6		
Pasteurized milk	2	0.8 -1.2		
Oysters	2	<0.35-0.7		
Soil	4	<0.5	<0.5	<0.5
Urine	3	<0.5	<0.5	<0.5
Water	17	<0.5 -0.7	<0.5	<0.5
Unknown	23	<0.5 -1.4	<0.5	<0.5

ency to higher values for strains of fecal or milk origin is of significance. The strains isolated from human urine showed low resistance.

DISCUSSION

According to Ayers and Clemmer (1918), fresh raw milk will not contain more than 2000 coliforms per ml. if milked into clean pails. Furthermore, of 14 samples of certified milk held at 60°F. (15.5°C) for twenty-four hours, none showed as many as 500 coliforms per ml. Inasmuch as these authors showed further that in milk kept at 50°F. or less (the recommended practice) there is practically no growth of coliforms, it may be assumed that in reasonably good grade milk which is to be pasteurized the colon counts should not exceed 10,000 per ml. With the recommendation of the Standard Milk Ordinance of the United States Public Health Service that pasteurization be for 30 minutes at 143°F., this means that the DRT of coliforms in raw milk under these recommended conditions must be at least 7.5 minutes in order that one or more organisms per ml. shall survive pasteurization. Even with 100,000 coliforms per ml. to start, a DRT of at least 6.0 minutes must obtain. At 142°F. (61°C.), these

times would be distinctly longer, in comparison with those found in our experiments.

These considerations, taken together with the above experimental data, lead one to suspect that very few coliform bacteria ever survive pasteurization. Thus, Ayers and Johnson (1913) report that no coliforms survived their experimental pasteurization of raw market milk. In 1915 these same authors published a study on pasteurization survival of colon bacilli (Ayers and Johnson, 1915) which is often quoted to show that coliform tests of pasteurized milk are meaningless. This is not necessarily a proper interpretation of the experimental findings. Ayers and Johnson report that of 174 cultures of bovine and dairy-product origin, 90 survived pasteurization at 140°F. and twelve survived at 145°F. Investigation of their experimental methods shows that they lack comparability with routine public health laboratory conditions. First, massive inoculations were used, not corresponding to expectations in good quality milk. Second, their test for sterility has no relation to the one usually used. This is demonstrated in certain of their experiments cited in detail. Starting at a concentration of five to six million bacteria per ml., flasks of milk containing strains "known to survive 145°F." were pasteurized for thirty minutes at 145°F. (62.8° C.) and cooled, then held at room temperatures for twenty-four hours. Plating tests showed only one organism in eight or nine ml. at this time. When the milk was kept at 8°C. (46°F.) it was essentially sterile even at nine days.

It therefore seems not unlikely that pasteurized milk showing numbers of coliforms has been subjected to post-pasteurization contamination. An interested agency can settle this question in any given case by simple heat-survival tests on the extracted organisms. Taken in conjunction with phosphatase tests, it should be possible to distinguish contamination from incomplete pasteurization or pasteurization survival.

The use of the DRT allows one to calculate minimum pasteurization requirements for milk containing various pathogens, if any data as to the concentration to be expected are available. It also allows the very important calculation of margin of safety in pasteurization processes. Thus, for example, if a given organism has a DRT of 5 minutes at 135°F., a DRT of thirty seconds at 145°F., and under the worst circumstances is not found in greater concentration than 100,000 per ml., the following margins of safety obtain, using one individual organism in ten ml. as the tolerance limit (pasteurization for thirty minutes at 145°F.):

Temperature margin of 10°F.;
Time margin of 27 minutes;
Concentration safety factor of 10^{54};
DRT variation safety factor of 10.

The tolerance limits in any given case will depend upon the relative infectivity of the organism concerned, and the amount of milk likely to be consumed by any given individual.

It is obvious that the more temperature-labile the organism and the lower its probable concentration in the milk, the greater the margin of safety. However,

in the practical case, it is necessary to judge the safety factor in terms of the pathogen most likely to survive the pasteurization treatment. This has always been taken to be the tubercle bacillus. Unfortunately it is not possible to calculate a DRT for this organism from the published data. Table 3 contains a

TABLE 3

*Decimal reduction times in milk calculated from data in the literature**

ORGANISM	TEST TEMP.	DRT†	CITATION
	°C.	min.	
Corynebacterium diphtheriae	32–55	<0.46	Rosenau, 1908
Corynebacterium diphtheriae	62–63	<4.5	Arnold and Gustafson, 1930
Shigella dysenteriae	55–60	<0.44–<0.61	Rosenau, 1908
Eberthella typhosa	55–60	<0.8 –<1.0	Rosenau, 1908
Eberthella typhosa	62–63	<4.5	Arnold and Gustafson, 1930
Aerobacter aerogenes	63	0.37	Sattler, 1928
Pseudomonas fluorescens	63	0.31	Sattler, 1928
Brucella abortus (cow)	62–63	<4.7	Arnold and Gustafson, 1930
Brucella abortus (caprine)	62–63	<4.8	Arnold and Gustafson, 1930
Brucella abortus (porcine)	59–63	5.4 – 7.3	Arnold and Gustafson, 1930
Brucella suis	63	4.5 – 5.0	Park, Graham, Prucha and Brannon, 1932
Brucella (mixed)	60	0.59– 0.79	Park, 1928
Brucella	61	0.39– 0.59	
Brucella	63	<.39	
Staphylococcus albus	63	0.80	Sattler 1928
Staphylococcus albus	62	<4.3	Arnold and Gustafson, 1930
Micrococcus sulfureus	63	4.2	Sattler, 1928
Streptococcus epidermidis	61–62	<4.5	Arnold and Gustafson, 1930
Streptococcus lactis	63	0.64	Sattler, 1928
Streptococcus ("25 mixed erysipelas strains")	59	<0.7	Park, 1928
Streptococcus (non-hemolytic food-poisoning type; cheese)	61	11.1	Linden, Turner and Thom, 1926
	63	4.5	
	62	19.4	Park, 1928
	63	<11.8	
Vibrio cholerae	28–53	<0.26–<0.81	Rosenau, 1908

* No corrections were attempted for colonial forms.

† DRT values given apply only to the experimental temperatures cited; the DRT values for Rosenau (1908) are upper limiting values for the highest temperature designated. < is used where the published data only allow estimation of upper limiting values for the DRT.

summary of some DRT values in milk near pasteurization temperatures calculated from the literature. The use of arbitrary times (Arnold and Gustafson, 1930) or continually varying temperatures (Rosenau, 1908) make precise determinations and comparisons difficult in some cases. The values of DRT for colonial forms may be only formal.

CONCLUSIONS

The use of the decimal reduction time offers a criterion for the determination of the margins of safety in the pasteurization process. It is equally valuable for evaluating any other processes which depend upon the thermal destruction of bacteria, where the order of death follows the monomolecular reaction rate. In view of these advantages it is suggested that the DRT be included as one of the standard criteria for determining the efficacy of pasteurization and similar processes.

SUMMARY

1. A study has been made of the decimal reduction times of 66 strains of coliform bacteria in milk at 61°C. (142°F.).

2. The experimental data indicate that few, if any, coliform bacteria survive pasteurization under ordinary circumstances, none of the DRT values being over 2 minutes in the strains tested.

3. The methods of determining the margins of safety of a pasteurization process are noted.

4. The DRTs of a number of other bacteria, for various temperatures, are tabulated. These are based upon values for thermal death in milk published by other workers.

5. It is suggested that the DRT principle be employed for the evaluation of all processes involving the thermal destruction of bacteria.

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