TYPES OF GROWTH RATES EXHIBITED BY HEAT RESISTANT ORGANISMS ISOLATED FROM MILK

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

Frank Robert Peabody

1948

THESIS

This is to certify that the

thesis entitled

Types of Growth Rates
Exhibited by Heat Resistant Organisms
Isolated from Milk

presented by

Frank Robert Peabody

has been accepted towards fulfillment
of the requirements for

M. S. degree in Bacteriology

Major professor

Date May 27, 1948

M-795

TYPES OF GROWTH RATES EXHIBITED BY HEAT RESISTANT ORGANISMS ISOLATED FROM MILK

by

FRANK ROBERT PEABODY

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Bacteriology and Public Health

1948

THESIS

ACKNOWLEDGMENT

The helpful suggestions and kind assistance
of

Dr. W. L. Mallmann

faculty member
of the
Department of Bacteriology and Public Health
of
Michigan State College

are greatly appreciated

OUTLINE

INTRODUCTION

The introduction of tryptone glucose extract agar as a standard plating medium in the Standard Methods for the Examination of Dairy Products (1) increased the total counts to a marked extent. This increase in count was due in part to the appearance of the thermoduric bacteria which were present only to a limited extent on the former standard agar. The result was a marked increase in counts of pasteurized milk from sources high in thermoduric organisms.

In order to maintain bacterial counts of pasteurized milk within the limits set by standard ordinances, it was necessary for the dairy to eliminate the thermoduric bacteria from the milk. Although the organisms are found on the dairy equipment, the chief source is producer milk. Accordingly, considerable research was conducted on this group of bacteria and standards were set by various research workers on the maximum numbers allowable.

Thus, it is current practice for milk sanitarians and others interested in dairy sanitation to make routine examinations for thermoduric organisms in milk as an aid in determining the causes of poor quality. The thermoduric count of milk is used as an indicator of the sanitary conditions of the equipment while high raw milk counts are supposedly due to faulty cooling. When a producer's milk shows a high incidence of thermoduric organisms, it is customary to make a field

inspection. Generally, the field man finds dirty equipment. Proper cleaning, coupled with effective cooling, produces a good quality milk. Such field observations have established the belief that the presence of thermoduric organisms are evidence of dirty equipment. Thus, it could be assumed that a low thermoduric bacteria count indicates good sanitation. The field man or sanitarian evinces little interest in the producers of low count milk.

One of the first things to be considered in any discussion of thermal effects on bacterial growth is a definition of terms. The term "thermoduric mesophile" was coined specifically to describe those organisms in milk which were found to be heat resistant. The expression does not necessarily indicate a time and temperature relationship or the ability of the organisms to reproduce at pasteurization temperatures. There appears to be no exact definition of the term "thermoduric" even though it is widely used in the literature.

In this thesis the term "thermoduric" will be used to designate all organisms surviving pasteurization irrespective of whether they merely survive or actually reproduce at a pasteurization temperature of 143° F. Gaughran (5) classifies thermophilic bacteria as those organisms which have an optimum temperature of 50° - 60° C. However, for convenience in presentation, no distinction will be made between thermoduric and thermophilic organisms except as indicated by actual growth curve data.

About three years ago, a field study of fifty producers was initiated by the Department of Bacteriology, the Lansing Department of Health and a local dairy to evaluate a number of different methods of sanitizing milking machines. The study was reported by Mallmann, et al, in 1946. (11). The program extended over a period of 12 months. Data were collected on the total counts of the raw milk and the thermoduric counts after laboratory pasteurization. These extensive data from which the above report was taken, offered an opportunity to study the correlation between sanitary practices and milk counts--both raw and thermoduric--under actual field conditions. The information was especially valuable since it was an unselected group of producers whose sanitary practices were being observed but not controlled.

One of the most striking features of this report is the irregularity with which high counts of thermoduric organisms are encountered. This is true of both the good and poor producers. For example, sporadically, a good quality milk with a low thermoduric count may show for a day an unusually high incidence of thermoduric organisms followed by a return to the low count. A high total count milk may have a low thermoduric count which still shows the same irregular increases in thermoduric indices as a good quality product. This is demonstrated by the data from two selected milk supplies representing a good and a bad producer. The total and thermoduric counts for several weeks are presented in Table I. (10).

Table I. A comparison of the records of a good producer and a poor producer expressed as organisms per ml.

Date	Producer "A"		Producer "B"	
	Total Count	Thermoduric Count	Total Count	Thermoduric Count
1/2	10,000	3,300		
1/10	20,000	5,000	38,000	600
1/24	12,000	11,000	100,000	2,900
1/31	31,000	4,200	250,000	800
2/7	19,000	6,000	1,700,000	800
2/21	21,000	2,900	140,000	27,000
3/7	32,000	1,100		
3/20	10,000	1,200	164,000	1,300
4/3	52,000	500	1,560,000	1,800
4/17			12,600,000	1,100
Logarithmic Average	19,950	2,740	476,300	1,740

Producer "A" had a consistently good quality raw milk with a logarithmic average count for this period of 19,950 organisms per ml. The record of Producer "B" was much lower in quality with the logarithmic average of 476,300 organisms per ml. for this period. However, in the case of the thermoduric counts the situation was quite different. In the record of Producer "A" the highest thermoduric count--almost twice as large as the second highest--is found in the same sample as one of the lowest total counts. And it also happens that the highest total count came from the same sample as the lowest thermoduric count. Examination of the data on Producer "B" gives even greater discrepancies. The wide variations in total counts are not reflected in the thermoduric counts. While the highest number of thermoduric organisms is found in one of the better samples, the three lowest thermoduric counts (which are essentially the same) came from samples having total counts of 38,000, 250,000 and 1,700,000 organisms per ml. It may also be noted that the average total count of Producer "B" is about 25 times that of "A", but the thermoduric counts are of the same order. In fact, Producer "A" shows the higher average thermoduric count.

Although these two are selected cases they are representative of other producers studied. It is not to be implied that all of the data are as striking as the instances cited, but these variations are of sufficient frequency to warrant serious consideration. Statistical analysis of these data now in progress may show similar trends between

the two but from a practical standpoint, these marked fluctuations and lack of correlation must be considered and explanations be found. In each of four groups of producers studied, the three best and three poorest were selected. The counts of the twelve producers in each of these classifications were averaged and the following results obtained:

	Organisms per ml.	
	Total count	Thermoduric count
Three poorest in each group	290,000	2800
Three best in each group	48,000	1100

These data show that the thermoduric counts are not essentially different for the two groups. This substantiates the observations made on the averages of the two producers with these data on 24 producers for a period of 12 months.

This all added up to the fact that there were many observations of the behavior of thermoduric organisms which could not be explained on the basis of known facts. Why does a supply of milk which has low counts suddenly show a high thermoduric count without a corresponding increase in the total count? Should the thermoduric counts parallel the total counts, should they remain fairly constant for all samples or are the two entirely independent? What explanation can be offered for one supply which has a high total count with a very low percentage of thermoduric organisms while another may be composed almost entirely of thermoduric bacteria?

The routine determination of thermoduric organisms in milk seemed to offer little help in solving these questions. It appeared that some fundamental studies on the thermoduric organisms, particularly as they pertained to growth patterns at various environmental temperatures, would be valuable. This thesis presents the results of a study planned to furnish some of this basic information on the thermoduric group of bacteria.

LITERATURE REVIEW

Many organisms have been isolated from pasteurized milk by various workers. These organisms are cited in detail in an excellent review by Robertson in 1927 (12). Both sporogenous and non-sporogenous types are represented. In the latter group are streptococci, micrococci, sarcinae and lactic acid bacteria.

Just prior to 1920, many laboratories doing control work on dairy products occasionally found small, so-called "pin-point" colonies. These were associated with pasteurized milk. Although several investigators had been working with these "pin-point" colonies, Dotterrer (4) was the first to recognize the fact that some of these organisms might be growing in the pasteurizer. The work on the "pin-point" colonies has been well reviewed by Tanner and Harding. (13).

Taylor was apparently the first to use laboratory pasteurization as a control measure. He reported, in 1924, that improperly sterilized

milk utensils were a source of thermoduric organisms. (14). Laboratory pasteurization was also used in 1931 by Hussong and Hammer. (9). They found no relationship between the initial counts and the pasteurization efficiency. Both high and low efficiencies were found with high count milk and also with low count samples. In some cases there was a tendency for high pasteurization efficiency on high count milk and a low efficiency on low count milk.

The regular use of laboratory pasteurization for control work on a large scale was introduced by a group of workers in the United Dairies, London, England. (2). They too, found considerable variation in tests made periodically on various farms. They concluded that different methods of sanitation were responsible. Contrary to the work of Hucker (8) who found little effect of holding temperatures for the first four hours, these workers found that a period of more than two hours would result in a rapid increase in heat-resistant bacteria.

These and other studies were reviewed by Hileman in 1940. (6). He also observed that several investigators found a large percentage of the flora of the udder was heat-resistant.

For a few years prior to the adoption of the tryptone glucose extract agar as a standard plating medium in the Standard Methods for the Examination of Dairy Products, (1) there were many papers on the effects of changing the standard agar. Typical is the report of Hileman, Moss and Stead (7) in which they showed that there was an increase in both the total and thermoduric counts and that the latter increased two

and one-half to five times as much as the former. A standard of 40,000 thermoduric organisms per milliliter was recommended by Bryan, Mallmann and Turney. (3).

EXPERIMENTAL PROCEDURE

The thermoduric cultures used in this study were isolated from plates made of routine raw milk samples which had been pasteurized in the laboratory. To obtain a variety of types, the forty cultures represent eighteen different producers. The distribution is shown in the following listings:

Culture Numbers	Producer
1 - 5	3
6 - 8	5
9 - 10	8
11 - 14	9
15	11
16	12
17 - 18	15
19 - 20	16
21 - 24	18
25 - 26	21
27 - 28	37
29 - 30	38
31	34
32 - 34	35
35	31
36	32
37 - 38	33
39 - 40	32

Although various colony types were selected on each set of plates, the 40 cultures do not necessarily represent 40 different species. Preli-

minary identification at the start of the experiments indicated that several cultures were closely related.

Inasmuch as the objective of these studies was growth rates, it was felt that elimination of related or similar types could be accomplished by comparative study of the growth rates.

To be sure that the cultures were resistant to pasteurization by the holding method, they were subjected to 143° F. for 30 minutes using the following technic:

Sterile milk was pre-heated in one-by-four inch test tubes and then 0.5 ml. of a broth culture or agar slant suspension was introduced into the tube. At the completion of the test period of 30 $\pm$ 0.5 minutes, the milk was plated out on tryptone glucose extract agar to determine the survival of each test organism. Thermostatically controlled water baths were used throughout which maintained the bath temperatures at 62° C. with a maximum variation of 1° C.

The results of the initial tests showed that all the cultures were capable of surviving this type of pasteurization. Near the completion of the studies, all the cultures were re-checked and found to have retained their resistance over the period of eighteen months.

It was found that the heavy pellicle formation of several broth cultures was very difficult to disperse. When these cultures were carried on agar slants, they were removed and readily suspended. The most satisfactory inoculum for the growth curves was obtained by transferring a small portion of the growth to a tube of broth. This was

immediately shaken vigorously and one ml. removed to a standard dilution blank containing 99 ml. of sterile, distilled water. One ml. was then used as the inoculum for the test.

The standard dilution bottle was utilized for the incubation flask. Sterile milk was used for the substratum so that the organisms could be tested in their natural environment.

The customary procedures involved in determining a growth curve were used. The incubation bottle containing about 100 ml. of sterile milk was pre-heated (or pre-cooled) to the temperature of the test and seeded with one ml. of the broth suspension, prepared as above. After the inoculum had been dispersed by the standard technic for shaking dilution bottles, the number of organisms per ml. was determined by a plate count according to the Standard Methods for the Examination of Dairy Products. (1).

The bottles were then placed in the incubator (or refrigerator) at the test temperature. Plate counts were made of the samples at varying intervals depending upon the organism and type of test. The length of time the bottles were removed for sampling was kept at a minimum and never exceeded five minutes.

Insofar as possible every effort was made to standardize conditions and control the variable factors. Temperatures of the incubators were recorded by either a continuously recording thermometer or a maximum-minimum type thermometer. With the high temperature incubator, the charts showed a variation of 5° C. The refrigerator

temperature was very constant with an occasional fluctuation of not exceeding 2° C. The 37° C. incubator showed a variation of not more than 3° C.

Agar plates were incubated for 48 hours at 37° C. before counting to eliminate the effect of any initial lag periods due to drastic, but sub-lethal treatment of the organisms in some of the tests.

All agar slants and agar plates were made with tryptone glucose extract agar (Difco). Other media were formulated from Difco ingredients. The maltose was passed through a Seitz filter and added to Purple Agar Base (Difco) aseptically.

The various cultural and bio-chemical characteristic of the organisms were determined. The cultures were all found to be Gram positive, facultative anaerobes which did not ferment lactose but produced an acid butt and alkaline slant on maltose agar. A rennet curd and reduction were present in all the litmus milk tubes. Differences were found in their morphology and reactions in dextrose broth which are given in Table II.

RESULTS

Because of the inability to obtain a constant inoculum when using different cultures, it is difficult to compare one curve to another on the basis of organisms per milliliter. Therefore, the results were reduced to units which were common to every curve. This was done by

Table II. Variable characteristics of the organisms used in this study.

Culture No.	Fermentation of Dextrose broth	Morphology
1	acid	cocci in pairs
2	acid	cocci in chains
3	none	cocci
4	none	short rods
5	none	cocci
6	acid	large cocci
7	none	cocci
8	none	rods - bipolar staining
9	none	cocci
10	acid	long, filamentous cells
11	acid	long rods
12	none	cocci
13	none	cocci
14	none	rods - bipolar staining
15	none	short, small rods
16	acid	cocci
17	none	cocci
18	acid	cocci
19	none	cocci, Staphylococcus-like clumps
20	none	cocci
21	none	rods - bipolar staining
22	none	cocci
23	none	
24	none	short rods - bipolar staining
25	none	rods in short chains
26	none	short rods - bipolar staining
27	acid	short, fat rods
28	acid	
29	none	rods
30	none	cocci
31	acid	diplococci
32	none	rods
33	acid	rods - filamentous
34	none	cocci in long chains
35	none	cocci
36	acid	cocci
37	none	cocci
38	none	cocci
39	none	cocci in packets of 4 or 8
40	none	cocci

calculating a "growth coefficient" by dividing the count in organisms per ml. at the initial sampling into the number of organisms per ml. at any other time.

$$\text{Growth coefficient} = \frac{\text{Organisms per ml. at "t" hours}}{\text{Organisms per ml. at 0 hours}}$$

This would give the results in numbers of times the culture had increased. Thus, a culture which increased from 120 to 12,000 organisms per ml. would be comparable to another which might have increased from 8000 to 800,000 organisms per ml. in the same period. It is recognized that there are limitations to the system. Since all of the ratios are dependent upon the initial count, a large error at this point is reflected inversely throughout the entire curve. If it were applied over too great a range, such factors as food supply and accumulation of toxic products would have to be considered. However, it does offer a means for the comparative study of the growth rates of a large number of heterogeneous cultures.

Growth Curves at 37° C.

Following the procedures previously outlined, growth curves were run at 37° C. over a period of 12 hours. The data arranged according to their increase at the end of the 12 hour period, are presented in Table III. The cultures may be divided into groups according to their rate of growth. The first five cultures are obviously capable of much more rapid multiplication at this temperature than are the other cultures.

Table III. Growth rates of a related group of thermoduric organisms at 37° C. expressed in growth coefficients.

Culture No.	Growth coefficients				
	0 hours	2 hours	4 hours	6 hours	12 hours
39	1	1.1	1.6	9.5	35,500
5	1	1.6	5.0	79.	18,000
2	1	3.0	44.	337.	12,000
38	1	.6	2.5	9.2	6,744
37	1	.7	1.6	10.5	2,100
36	1	.9	1.7	21.	738
29	1	1.3	2.2	3.6	410
16	1	1.2	1.4	1.9	387
35	1	1.4	.9	2.2	341
30	1	1.1	2.1	5.4	337
32	1	.4	4.0	26.	333
27	1	1.4	2.0	5.2	253
21	1	1.2	2.4	4.5	172
26	1	1.4	2.4	5.8	129
7	1	1.1	1.7	2.7	128
12	1	.9	.8	2.5	126
28	1	1.2	2.2	2.6	114
17	1	2.0	2.3	11.	71
20	1	1.5	2.8	10.	66
9	1	-	.5	5.0	60
22	1	1.0	1.8	2.5	49
19	1	.7	1.1	1.1	45
18	1	1.8	2.5	24.	32
10	1	.8	1.1	1.5	24
40	1	.4	1.5	4.2	23
31	1	-	4.0	2.0	15
14	1	1.3	1.3	1.2	14
25	1	1.2	1.4	2.6	13
33	1	1.9	1.6	2.2	12
8	1	1.1	1.6	2.3	9.3
11	1	.5	1.7	2.6	8.0
4	1	.9	-	1.5	7.7
3	1	1.5	1.6	.3	6.8
34	1	.6	1.5	1.5	6.6
15	1	1.0	1.4	1.4	3.4
13	1	1.7	1.3	1.3	3.3
1	1	.8	.8	.8	1.7

The second group might be considered as those which increased at least 100 times but less than 1000 times in twelve hours. This would include the next twelve cultures.

The remaining cultures fall into two groups--those exhibiting a slow rate of growth and the last few which show essentially no growth at this temperature. It is difficult to state exactly where this distinction should be made. Arbitrarily, a culture which increases less than five times in a given period would be classed as "no growth."

On the basis of this table, it is evident that a group having such a range of growth characteristics would be likely to give diverse results depending upon the members of the group which are present. These curves represent data which were obtained at a temperature not ordinarily encountered in dairy practice. For that reason another series of growth curves was carried out.

Growth Curves at 25° C.

In order to more closely approximate improper cooling and storage, the complete series of cultures was tested at 25° C. The data are presented in Table IV. Because of the longer lag phase and the slower rate of growth at this temperature, the tests were extended to 24 hours to obtain a wider range in the results. Again using the classifications of the first series, the cultures show the same breakdown into groups according to their growth rates. The extremes are not as great as those obtained at 37° C. It would be expected that some of them

Table IV. Growth rates of a related group of thermoduric organisms at 25° C. expressed in growth coefficients

Culture No.	Growth Coefficients				
	0 hours	3 hours	9 hours	12 hours	24 hours
38	1	2.1	5.2	7.4	4,680
26	1	1.1	3.1	16.	3,000
29	1	1.5	6.1	17.	2,780
30	1	1.2	2.0	3.0	2,250
32	1	1.5	2.2	10.	1,300
17	1	1.0	1.8	1.8	1,210
31	1	1.1	3.4	8.9	890
25	1	2.5	3.2	10.	660
20	1	.4	1.7	1.2	620
18	1	1.2	1.2	1.2	596
16	1	1.7	2.9	6.4	573
22	1	1.1	1.3	1.6	530
21	1	.7	1.3	1.6	440
24	1	2.0	1.5	6.0	400
8	1	4.0	8.0	10.	400
13	1	1.1	2.3	10.	230
37	1	1.1	11.	23.	180
27	1	1.2	2.1	6.5	178
5	1	2.2	3.2	12.	143
4	1	1.8	3.1	6.7	141
2	1	1.3	1.3	2.7	135
28	1	1.6	3.3	13.	125
12	1	4.6	-	16.	115
7	1	.9	3.2	3.8	100
6	1	1.1	1.4	4.9	88
19	1	.7	1.0	1.0	87
1	1	.7	1.1	1.5	72
14	1	1.1	1.6	5.9	59
11	1	.8	1.1	3.1	45
9	1	1.1	1.8	6.4	40
3	1	1.4	2.4	9.2	34
39	1	.9	2.3	4.9	26
40	1	1.0	2.7	2.9	24
34	1	1.1	2.1	4.4	18
35	1	1.3	5.4	-	16
33	1	1.7	1.8	4.8	10
10	1	1.2	.6	1.6	5
36	1	.6	1.2	1.5	4

would grow much more rapidly at 37° C. than at 25° C. Although the relationship of the various temperatures will be discussed later, it may be pointed out here that the cultures do not occur in exactly the same order in both sets of data. This change in temperature will have different effects on different members of the group of thermoduric organisms--presumably due to different optimum temperatures for growth.

The above data indicate that the optimum temperature for growth of some of the organisms may be higher than 37° C. Tests were made at higher temperatures to determine, if possible, the approximate limits of growth.

Growth Curves at 51° - 55° C.

From the cultures tested a set of nine, based on growth rates at 37° C., was chosen to represent the different types for similar studies at 51° - 55° C. The results are presented in Table V. At this temperature the differentiation was very marked. Three of the cultures showed only a slight increase, if any, followed by a rapid decrease. However, the other six cultures, gave quite rapid growth. In some cases they reproduced rapidly and reached the death phase of the growth curve by 24 hours and in others this stage was not reached until the third day.

Some of the thermoduric organisms had growth limits below 50° C. as indicated by little or no increase in numbers during the test. Others, however, were capable of more rapid growth at 50° - 55° C.

Table V. Growth rates of a selected group of thermoduric organisms at 51° - 55° C. expressed in growth coefficients.

Culture No.	0 hours	3 hours	6 hours	12 hours	24 hours	2 days	3 days
13	1	8.2	6,530	20,000	6,070		9,300
38	1	32.	5,900	6,250	1,950		3,200
16	1	21.	2,130	1,600	4,350		1,600
20	1	21.	2,200	2,200	142		615
19	1	9.7	546	5,600	750		4.8
15	1	7.7	755	2,900	500		2,700
4	1	2.6	2.7	.6	.5	.2	.1
12	1	.8	.6	.5	.1	0	
9	1	4.	1.4	1.4	0		

than at 37° C. and might even be classified as thermophiles if their optimum were found to be in this range. Higher incubation temperatures were indicated for this group.

Growth Curves at 61° - 65° C.

The temperature of pasteurization was selected for the next series of studies. Eight of the cultures tested at 51° - 55° C. were selected for this series. The incubator used was equipped with a recording thermometer which indicated that the fluctuation of the air temperatures was from 61° to 65° C. in regular, even cycles of about one hour. Therefore, the temperature of the milk must have remained very close to the 62° mark and actually approximated very closely pasteurization for an extended period.

An examination of the data in Table VI shows that none of the cultures were able to increase in numbers at this temperature since the growth coefficients are all less than 1. It does indicate that several of them are resistant to the temperature of pasteurization for eight hours or more.

Growth Curves at 5° C.

To determine the effect of proper storage conditions, growth studies were made at 5° C. for 3 days. The same set of cultures used for the experiments at 51° - 55° C. and 61° - 65° C. was used in this series. An examination of the data in Table VII reveals that these cultures decreased in numbers with two exceptions. In these the increase

Table VI. Growth rates of a selected group of thermoduric organisms at 61° - 65° C. expressed as growth coefficients.

Culture No.	Growth Coefficients						
	0 hours	3 hours	8 hours	16 hours	30 hours	2 days	3 days
38	1	.06	.005	.005	0	.005	0
9	1	.007	.007	.003	.003	0	0
15	1	.02	.01	0	.02	0	0
20	1	.04	.01	0	0	0	0
12	1	.62	.005	0	0	0	0
19	1	.09	0	.05	0	0	0
13	1	.04	0	0	0	0	0
16	1	.009	0	0	0	0	0

Table VII. Growth rates of a selected group of thermoduric organisms at 5° C. expressed as growth coefficients.

Culture No.	Growth Coefficients					
	0 hours	5 hours	12 hours	30 hours	2 days	3 days
9	1	1.1	1.15	.97	.71	.65
12	1	1.1	.73	.26	.15	.12
19	1	1.12	.62	.30	.15	.07
4	1	.87	.66	.25	.20	.15
2	1	.75	.54	.27	.09	.07
15	1	.76	.50	.22	.09	.05
38	1	.79	.67	.20	.09	.05
13	1	.87	.62	.16	.11	.06
16	1	.91	.26	.07	.03	.02
20	1	.55	.19	.06	.04	0

was very slight and of short duration. It is evident that proper storage of milk is an effective method of preventing the multiplication of thermoduric organisms.

Correlation of the Growth Curves

Examination of these sets of data bring out several facts which are of interest. In the group of thermoduric organisms tested, it is found that a wide variation in growth rates of the different organisms occurred at all temperatures. In addition, each culture showed entirely different growth patterns at different temperatures. In Figures 1 and 2 are shown graphically the effect of temperature on the growth rates of two different organisms at the various temperatures tested.

A comparison of Tables III and IV shows that the order of listing of the cultures has changed. Close inspection will reveal that the majority have not changed from one extreme to another, but have shifted only slightly. In a few cases there is little change. This is due to the difference in optimum temperatures which will cause one organism to grow more rapidly under one set of conditions whereas another will have an increased rate under different conditions.

In general, the results show the great diversity of growth activity encountered with this group of thermoduric organisms.

Figure 1. Growth Curves of Culture 13

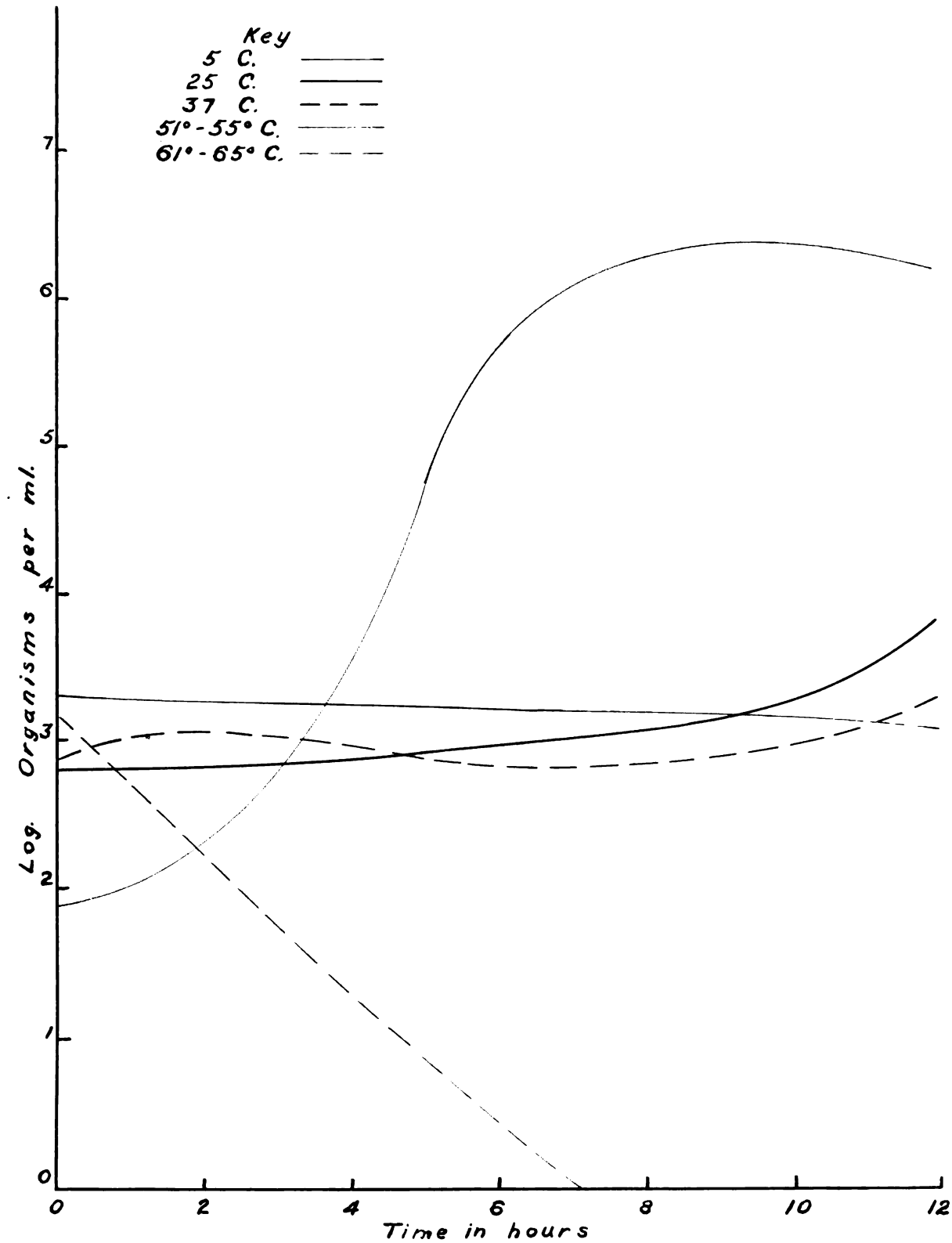
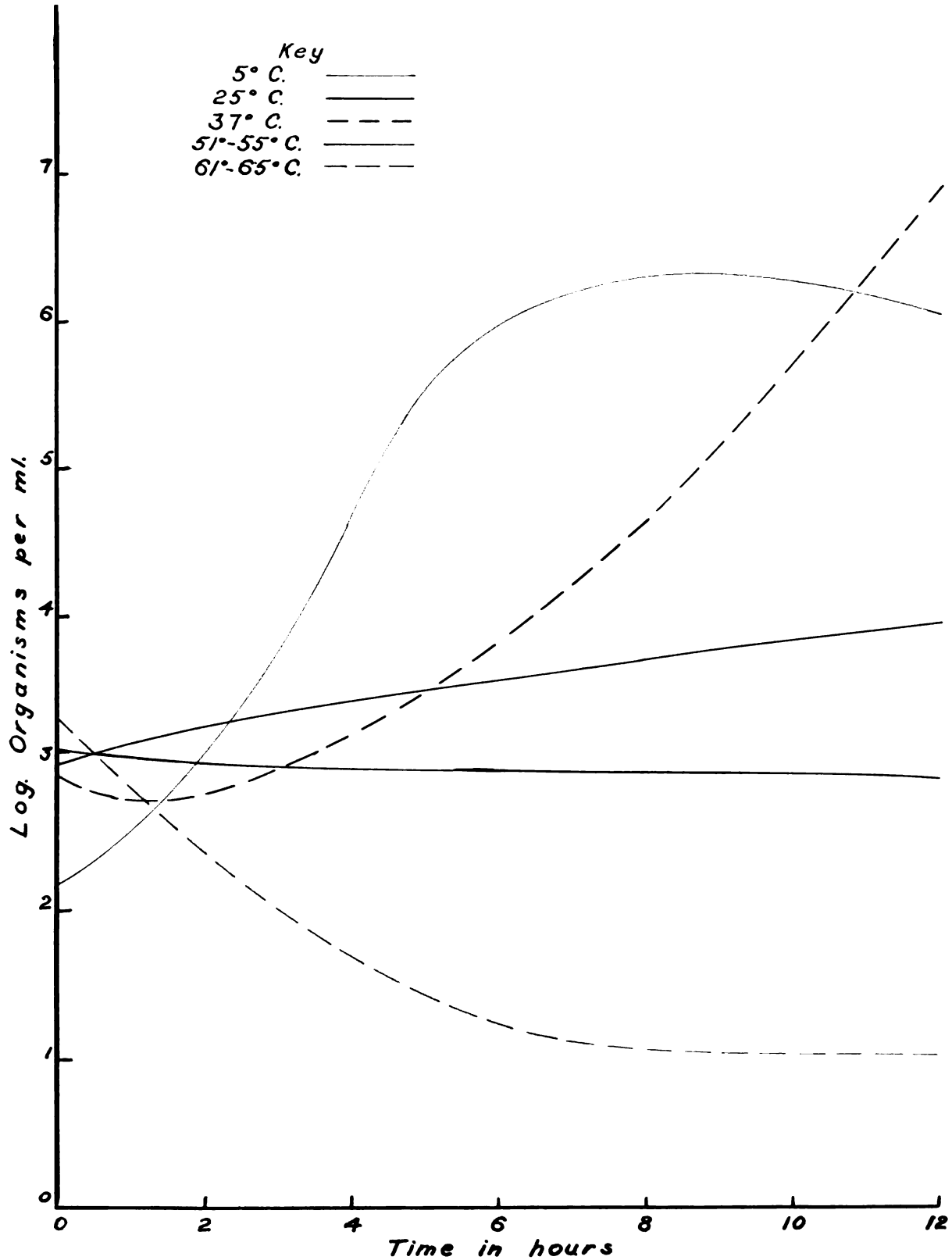


Figure 2. Growth Curves of Culture 38



DISCUSSION

The data presented show that it would be impossible to make any general statements on the behavior of the thermoduric group as a whole because a number of different growth patterns were evident in the unselected group of organisms tested. However, the data show that the growth curves at the various temperatures used in this study make possible the division of the cultures into three general groups; namely, (1) those whose rate of growth is very rapid, (2) the group with a moderate rate of growth (an increase of a few hundred times), and (3) the organisms that will reproduce slowly, if at all.

Even assuming the lack of any distinct separation of these groups if a large number of cultures were studied, these three general types of response would still be found.

Since it is impossible to obtain a bacteria-free milk, there is no reason to assume that a producer will get the same type of organisms contaminating his milk every day even though there is no difference in the degree of cleanliness of his equipment. If a good sanitation program is being followed, the total count should be low and fairly constant. However, this does not mean that the flora of the milk would remain the same. The variety of contaminants is almost infinite and any one, or several, might supply the predominating organisms for that milking. If there is a lack of adequate sanitation, the possibilities of variation would be increased proportionally.

Assuming that a thermoduric organism with a high rate of growth at 25° C. contaminates the milk, the count would increase many times during improper overnight storage. If the total count were initially high, both the total and thermoduric counts would be proportionally high when the milk reached the receiving station. On the other hand, if the initial count were low and predominately thermoduric, the total and thermoduric counts would be of the same order. If the thermoduric organisms had a low rate of growth at 25° C., the total count might be high but the thermoduric count would remain low.

Many combinations of the various types of thermoduric organisms could result. For example, in the case of Producer "A", referred to in Table I, it is conceivable, that one of his sources of possible contamination was largely thermoduric organisms. On April 4, the source of contamination was apparently of non-thermoduric types which were readily removed by pasteurization.

With Producer "B", the contamination seems to be mainly of non-resistant bacteria. One exception to this is the sample taken February 21. Since the trend of the thermoduric counts is low, it might be assumed that the general flora of this farmer's equipment and barn are predominately non-resistant to heat. Perhaps this particular instance was caused by poor cleaning of the equipment on the previous day and thermoduric organisms which reproduced fairly well at 25° C. were not removed. During storage of the equipment, considerable

multiplication could occur, and a high incidence of thermoduric organisms in the milk would result.

The data on the 5° C. incubation show that none of the cultures studied were capable of reproduction at a rate such that they would materially increase in numbers during proper storage. Further, three cultures which had been pasteurized were held at 5° C. for a period of six weeks. There was no gross evidence of change in the milk, but removal to room temperature for a period of 24 hours showed the usual curd formation and evidence of normal growth.

The studies of growth rates presented in this thesis are particularly important because they show that the incidence of thermoduric organisms in a milk supply is not entirely the result of the initial contamination of the milk. It is apparent that their numbers may be the result of initial contamination coupled with multiplication during improper storage. Because various thermoduric organisms show different growth rates at various incubation temperatures, it cannot be assumed that their presence necessarily indicates dirty equipment or their absence indicates clean equipment.

These laboratory studies indicate the need of carefully conducted field studies of both properly and improperly cleaned equipment to resurvey the significance of thermoduric bacteria in milk. Such studies are planned as a future project.

CONCLUSIONS

The thermoduric organisms studied showed a wide variation in their individual rates of growth.

On the basis of the growth rates, these cultures may be classified into three groups--those showing rapid growth rates, those with moderate rates of growth and those which show very little or no increase in numbers.

The use of proper cooling is an effective means of preventing the multiplication of organisms of the thermoduric type.

LITERATURE CITED

(1) American Public Health Association. Standard Methods for the Examination of Dairy Products. 7th Ed. 1939.

(2) Anderson, E. B. and L. J. Meanwell. "Studies in the Bacteriology of Low-Temperature Pasteurization. Part I. The Significance of Heat-Resisting Organisms in Raw Milk Supplies." J. Dairy Res. 4: 213. 1933.

(3) Bryan, C. S., W. L. Mallmann and G. J. Turney. "Some Microscopic Technics for Determining the Bacteriological Quality of Milk." Mich. State Coll. Agr. Exp. Sta. Circ. Bull. 186. 1944.

(4) Dotterrer, W. D. "Some Observations on High Counts in Milk Freshly Pasteurized under Commercial Conditions." Intern. Assoc. Dairy and Milk Inspectors Ann. Rpt. 12: 204 - 214. 1923.

(5) Gaughran, E. R. L. "The Thermophilic Microorganisms." Bact. Rev. 11: 189. 1947.

(6) Hileman, J. L. "Thermoduric Bacteria in Pasteurized Milk. A Review of the Literature." J. Dairy Sci. 23: 1143 - 1160. 1940.

(7) Hileman, J. L., Clarence Moss and Betty Stead. "Thermoduric Bacteria in Milk. III. The Effect of Changing Agar and Temperature of Incubation for Plate Counts on the Problem of Thermoduric Bacteria in Milk." J. Dairy Sci. 24: 799. 1941.

(8) Hucker, G. J. "Studies on the Coccaceae. VIII. A Study of the Cocci Resisting Pasteurization Temperatures." N. Y. State Agr. Exp. Sta. (Geneva) Tech. Bull. 134. 1928.

(9) Hussong, R. V. and B. W. Hammer. "The Pasteurization Efficiencies Secured with Milk from Individual Farms." Iowa Agr. Exp. Sta. Bull. 286. 1931.

(10) Mallmann, W. L. Unpublished data.

(11) Mallmann, W. L., Edgar Kivela, A. L. Bortree, Elbert Churchill and L. H. Begeman. "The Influence of the Method of Sanitizing Milking Machines on the Bacterial Content of Milk." N. Y. State Assoc. Milk Sanitarians Ann. Rpt. 20: 177 - 190. 1946.

(12) Robertson, A. H. "Thermophilic and Thermoduric Micro-organisms, with Special Reference to Species Isolated from Milk. I. Review of Literature." N. Y. State Agr. Exp. Sta. Bull. 130. 1927.

(13) Tanner, F. W. and H. G. Harding. "Thermophilic Bacteria from Milk." J. Bact. 11: 96. 1926.

(14) Taylor, G. B. "Some Observations on the Scientific Control of Milk Plants from the Standpoint of Bacteria." Intern. Assoc. Dairy and Milk Inspectors Ann. Rpt. 13: 287. 1924.

[illegible] USE ONLY Sum 24 48

Aug13 '54