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Influence of growth temperature on the thermal resistance of some aerobic, spore-forming bacteria from evaporated milk

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NOTE TO USERS

INFLUENCE OF GROWTH TEMPERATURE ON THE THERMAL RESISTANCE OF SOME AEROBIC, SPORE-FORMING BACTERIA FROM EVAPORATED MILK

by

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Outline

INTRODUCTION

The heat treatment employed in manufacturing evaporated milk ordinarily is satisfactory for the destruction of the microorganisms present. Occasionally, spoilage occurs even though there has been no intentional or discernible modification of the heat treatment used with the milk. The spoilage outbreaks are frequently spasmodic but during their brief existence may cause considerable financial loss to the manufacturer.

In connection with various outbreaks of spoilage in evaporated milk it has been observed at the Iowa Agricultural Experiment Station, and elsewhere, that the outbreaks often occurred during warm weather. It is entirely possible that the outbreaks were due to an extensive contamination of the milk, with the causal organisms, on the farm or in the plant since the warm weather may have been more favorable for the growth and development of the organisms. During warm weather there is also a greater opportunity for the contamination of milk because of the ease with which dry particles of soil, dirt, etc. containing organisms or spores can be carried in the air. An extensive contamination increases the difficulty of sterilization because of the large numbers of organisms present. Another explanation may be that if the causal organisms are thermophilic they would not grow and develop at

ordinary temperatures, even if present, but would grow at summer heat, thereby causing spoilage primarily at that season. From the suggestions advanced the importance of growth temperatures on the organisms responsible for spoilage in evaporated milk is apparent.

With the importance of growth temperature in mind, the hypothesis has been advanced that certain strains of bacteria causing spoilage in evaporated milk may be capable of withstanding more severe heat treatment when grown at relatively high temperatures. This possibility, together with the frequency with which outbreaks of spoilage have occurred during hot weather, makes important the evaluation of the influence of increased growth temperature on the thermal resistance of the bacteria most frequently present in evaporated milk. The microorganisms commonly found responsible for spoilage in evaporated milk are aerobic, spore-forming bacteria as reported by Hammer (36, 38), Hammer and Hussong (41), Hussong and Hammer (44), Kelly (45), Morrison and Rettger (53), Spitzer and Epple (76), and others.

STATEMENT OF PROBLEM

The primary interest of the problem is centered around the question -- what influence does growth temperature have on the heat resisting ability of aerobic, spore-forming bacteria found in evaporated milk? Data on this question may furnish at least a partial explanation for the heat resisting ability of bacteria which frequently cause serious spoilage losses in evaporated milk.

The work carried out was divided into the following sections:

A. Comparison of heat resistance of spores suspended in skimmilk and in evaporated milk.

B. Thermal resistance of spores of various bacteria grown at two different temperatures.

C. Thermal resistance of spores of various bacteria grown at three different temperatures.

D. Influence of a sudden change in growth temperature on the thermal resistance of spores of various bacteria.

E. Effect of continued growth of various bacteria at a changed growth temperature on the thermal resistance of the spores.

F. Comparative thermal resistance of moist and freshly dried spores.

G. Influence of age on the thermal resistance of dried spores.

H. Effect of continued growth of various bacteria on artificial culture media on the thermal resistance of the spores.

I. Relation of thermal resistance to growth and exposure temperatures.

J. Examination of normal samples of evaporated milk.

K. Thermal resistance of some aerobic, spore-forming bacteria isolated from raw milk.

L. Identification of the bacteria used in the study.

REVIEW OF LITERATURE

The literature relating to the heat resistance of bacteria is voluminous and covers many factors. For the purpose of brevity and continuity the literature reviewed is grouped under the following heads:

A. Theories of cell destruction by heat.

B. Factors influencing heat resistance of vegetative cells and spores.

1. Moisture content
2. Time and temperature of exposure
3. Hydrogen-ion concentration
4. Osmotic pressure
5. Acclimatization
6. Protective action
7. Age of culture
8. Resistance of spores
9. Numbers of spores
10. Growth conditions

C. Spoilage in evaporated milk.

Theories of cell destruction by heat

Relative to the definition of death, Rahn and Barnes (60) stated that, "Death is defined in many different ways, and always by the loss of some property characteristic to the living organism. Compelled by the technique, bacteriologists usually define a cell as dead when it has lost permanently the power of reproduction, - - other biologists usually consider a cell dead when it has lost the power of respiration (or fermen-

tation) or the loss of plasmolysis or by adsorption of dyes."

Various theories have been advanced to explain the processes involved in the destruction of bacteria and other cells by heat.

Chick (16), working with proteins, concluded that the destruction of cells by heat consisted of a reaction between the water and some protein essential for life, the reaction resulting in coagulation of the protein.

It was believed by Weiss (88) that the destruction of spores by heat was probably due to a gradual coagulation of the protein.

Mayer (51) concluded that death from high temperatures was due to accumulation of acid in the tissues (probably H_2CO_3).

Rahn (58) conceived of the cell as having one or more sensitive molecules which must be destroyed before the cell is killed.

Spaeth (75) believed that death of the cell was always due to factors which caused an increased permeability of the cell wall. Zoond (92), working with B. cereus, confirmed the results of Spaeth and showed that the death of the cell by heat was accompanied by an increase in permeability of the cell wall which allowed free diffusion of salts of the cell.

Buchanan and Fulmer (11) stated: "The death of a cell is probably due to some irreversible change in the proto-

plasm which has proceeded to such a point that it can not function. It may in some cases be attributed to coagulation of proteins, or to destruction of essential enzymes. It should be emphasized that death induced by high temperatures is due to the acceleration of chemical or physical changes which proceed more slowly at lower temperatures."

The theories of death by heat are summarized as follows by Rahn (59): "Death by heat shows such close analogies to the heat coagulation of proteins, in temperature range as well as in the high temperature coefficients, that the general explanation of death by heat being due to coagulation of some parts of the protoplasm seems well founded. The greater resistance of dry cells corresponds well with the absence of coagulation of proteins when heated in dry condition; dry bacteria at high temperatures do not die from coagulation, but from oxidation."

The growth and survival of a bacterial cell is extremely intricate and dependent upon a multiplicity of factors even under "normal" conditions of growth. When high temperatures are applied the study becomes more complicated since heat speeds up reactions. It is, therefore, unlikely that any one theory could be proposed that would cover cell behavior under all conditions, since, as pointed out by Miller (52), the cell protoplasm is itself complex in nature.

Factors influencing heat resistance of vegetative cells and spores

Moisture content. It is generally recognized by bacteriologists that the thermal resistance of a bacterial cell is dependent to a great extent on its moisture content. Hellmich, according to Lewith (48), isolated heat coagulable albumin from cell contents of bacteria. Lewith (48), in 1890, demonstrated that the temperature of coagulation of pure albumin was inversely proportional to its water content, and hence that hot water and steam owed their efficacious properties to the dilution of the protein material along with the heat.

Both Cramer (17) and Benecke (5) found the moisture content of spores of bacteria lower than that of the vegetative cells. Cramer (17) suggested that the resistance of bacterial spores to dry heat depended upon the retention of hygroscopic moisture by the spore wall, while the resistance to moist heat depended upon the retarding effect which the spore wall exerted on the penetrating moisture as it passed through to the albumin.

Burke (14) indicated that the degree of permeability of the cell walls of spores to water has an important influence on their resistance to heat, since the unusual heat resistance of a few individual spores which survive the majority thermal death point undoubtedly depends upon the greater impermeability

of the spore walls to water.

Robertson (63) concluded that the ability of bacterial cells to survive high temperatures depended, at least in part, upon a low moisture content.

Time and temperature of exposure. It is apparent that, in any one medium, the destruction, thermal death rate or thermal death time, of a definite number of bacteria under standardized environmental conditions depends on two factors, time and temperature of exposure. The thermal death point in one medium, however, may be appreciably different from that in another, owing to the influence exerted by many factors.

In general, the time necessary for sterilization varies inversely with the temperature as was found by Bigelow and Esty (8) when working with a known suspension of bacterial spores in a medium of a known pH.

Esty and Williams (29) very succinctly summarize their results when they stated: "The heat resistance of bacterial spores is not a constant but depends upon a large number of variables. Any statement regarding it should include all the conditions under which the spores are produced, heated, and subcultivated."

Bigelow and Esty (7) also found that lowering the temperature 10°C. increased the time necessary for sterilization about ten times.

Hydrogen-ion concentration. Weiss (88), working with the spores of Bacillus botulinus, found that the pH of the growth medium very materially influenced the thermal resistance of the spores. He concluded that both the H-ion and the OH-ion lowered the thermal resistance of the spore and the rate of this reduction decreased as either the H- or OH-ion concentration increased. Bigelow and Esty (8) also found the time necessary to destroy known suspensions of spores decreased as the pH value increased. Myers (55), working with Bacterium coli, found that an increase in the pH, on the alkaline side of neutrality, of a given solution increased its power to destroy the organism at a given temperature. Dickson, Burke, and Ward (22), by the addition of 5 per cent lemon juice to a medium, lowered the thermal death point of Bacillus botulinus. Buchanan, Thompson, Orr, and Bruett (12) also indicated the importance of the pH on the heat resistance of spores.

Osmotic pressure. Robertson (63) demonstrated that an increase in osmotic pressure was accompanied by a rise in the thermal death point of bacterial cells. He found hypotonic solutions decreased the resistance of cells and hypertonic solutions increased, within limits, the heat resistance. Other investigators have secured similar results, and their work will be discussed and elaborated on under the section discussing protective action.

Acclimatization. Both heating and drying have been used as a means of modifying the virulence of organisms; for example, the work of Dozier (23) and Starin (77) with Clostridium botulinus, and Wadsworth and Kirkbride (85) with the pneumococcus and the attenuation of the anthrax bacillus and rabies virus. It thus appears that there is a possibility of microorganisms acclimatizing or adapting themselves to resist higher temperatures. Magoon (50), by selection, developed a strain of Bacillus mycoides with a greater resistance to heat than that of the parent strain.

van der Sluis (82) observed that cultures of Bacterium tuberculosis in media containing milk acquired an ability to withstand a higher temperature than Bacterium tuberculosis normally present in milk.

Borman (9), working with strains of Escherichia coli, found that they acquired resistance to the bacteriostatic and germicidal action of cations and also that this acquired resistance towards one cation induced a comparable resistance toward other cations with different chemical properties.

Casman and Rettger (15) attempted to acclimatize members of the "subtilis group" by gradually raising the temperature of incubation. After one year only a negligible degree of success was apparent.

Robertson (63) believed that if certain microorgan-

isms had the ability of adaptation to higher temperatures the process of adaptability depended upon the elimination of water from the cell contents.

The work of Kelly (45), showing that atypical strains of Bacillus cereus, Bacillus simplex, and Bacillus megatherium were the causative agents in three types of spoilage in evaporated milk, suggests a possible confirmation of the contention that spoilage in evaporated milk may be due to super-resistant variants of common forms.

Protective action. It has been suggested by various investigators that an important factor aiding a microorganism in resisting destruction by heat is the existence of a protective coating about the cell. This may be an inherent part of the microorganism, such as a mucoid secretion or a capsule, or it may be an artificial covering, such as is formed when bacteria are heated in milk. Robertson (63) also suggests the dehydrating influence of hypertonic solutions.

Smith (73), working with the tubercle bacillus, was the first to observe that milk afforded a protective action to microorganisms. Russell and Hastings (65) confirmed Smith's observations. Ficker (31), Wolff (91), Barthel and Stenstrom (3), Gorini (33, 34), Ayers and Johnson (2), Holman (42), and Brown and Peiser (10) have also demonstrated the protective action of milk.

Watkins and Winslow (86) made conjectures as to the production, by cultures of Escherichia coli, of protective substances which increased the heat resistance of older cultures.

Winslow and Brooke (90) reported that meat extract and peptone increased the heat resistance of bacteria, perhaps by acting as a protective colloid. Sommer (74) and Murray and Headlee (54) also found that peptone possessed a protective action.

The protective action of sugar in increasing the heat resistance of bacteria has been shown by Anzulovic (1), Weiss (89), Robertson (63), Toulouse (80), and Fay (30) among others.

Viljoen (83) secured a marked protective action for bacteria against heat by the addition of 1.5 to 2.5 per cent NaCl to pea liquor.

Bartlett and Kinne (4), Bullock (13), and Dreyer and Walker (24), found glycerol and other waterfree fluids afforded bacterial cells protection against heat.

Esty and Meyer (28) heated Bacillus botulinus spores in the juices of 17 varieties of canned food and secured a variation in heat resistance of the spores of from less than 10 minutes to 230 minutes at 100°C.

Age of culture. Various investigators have found that spores or cells from young cultures are much less resistant to high temperatures than those from older cultures.

Dickson et al (21) and Magoon (49) reported old spores more resistant to heat than young spores. Stark and Stark (78) found the young cells of Streptococcus fecalis markedly less resistant than the mature cells. Weiss (88) and Esty and Meyer (28) found young moist spores were more resistant to heat than old spores.

Sherman, Stark, and Stark (72) stated: "--Some important milk bacteria (e.g., ropy milk types) may be eliminated from milk by pasteurization when the cells are in a young and growing condition, while the old cells of the same organism are able to withstand the process."

Sherman and Albus (70) noted that the young cells of Escherichia coli were more rapidly destroyed by heating to relatively low temperatures than were the older cells. Sherman and Cameron (71) reported that young cells of Escherichia coli may be killed by abrupt environmental changes within the natural range of growth of the organism.

Relative to non-spore-forming bacteria Robertson (64) said, "The young, rapidly growing or adolescent cells are more susceptible than the older cells to the killing action of high temperatures." Hammer and Hussong (40) reported similar results with Aerobacter aerogenes.

Resistance of spores. Burke (14) and Esty(27) observed the presence of especially heat-resistant individuals in spore suspensions. Shanley (69) noted variation in

the resistance of spores of different strains of the same species. Dickson et al (21) found marked variations in the resistance of spores of Clostridium botulinus. Magoon (49) stated, "The resistance of spores to heat is not a fixed property but a variable one, the degree of resistance being influenced by age, the temperature and humidity of the environment, and possibly other factors."

Number of spores. There is considerable experimental evidence showing that with an increase in the size of the initial seeding there is an increase in the length of time required for complete cell destruction or sterilization. This fact is noted in the work of Eijkman (26), Bigelow and Esty (8), Esty and Meyer (28), Esty (27), and Watkins and Winslow (86).

Growth conditions. Some investigators, such as Rabinowitsch (57), Schillinger (68), and Tsiklinski (81), have suggested that unusual thermal resistance can be acquired by the aerobic spore-forming species. Eckelmann (25) showed that the cultures she studied had the ability to partially lose or recover their heat resistance. Growth in liquid media decreased the heat resistance, but long growth on solid media resulted in recovery of the heat resistance. Curran (19) found the heat resistance of spores formed and held on artificial media was less than that of those formed and held in a natural environment.

von Esmarch (84) and Gruber (35) showed that the resistance of microorganisms was partly dependent upon the conditions under which they had been grown. Weil (87) confirmed the work of von Esmarch and noted that the temperature of incubation appeared to influence the ability of anthrax spores to resist heat.

That the type of culture medium used influences the heat resistance of microorganisms has been demonstrated repeatedly, notably by Weiss (88), Reiter (61), Esty (27), and Watkins and Winslow (86). Esty and Meyer (28) grew Bacillus botulinus in various media and found the heat resistance varied widely.

Spoilage in evaporated milk

According to Lawrence and Ford (46), Hueppe was the first to call attention to the presence of aerobic, spore-forming bacteria in milk. Flügge (32), in 1894, described 11 species found in boiled milk. Since then most of the investigators studying thermophilic, aerobic, spore-forming bacteria have shown them to be present in milk. Some of the workers have been Leichmann (47), Rabinowitsch (57), Schardinger (67), Tanner and Harding (79), Lawrence and Ford (46), and Prickett (56). Since thermophilic, aerobic, spore-forming bacteria are usually present in milk, although milk is not their normal habitat, it is possible that the aerobic, spore-forming

bacteria found in spoiled evaporated milk come from the original raw milk.

Aerobic, spore-forming bacteria found to be responsible for spoilage in evaporated milk are: Bacillus coagulans by Hammer (36); Bacillus amarus by Hammer (38); Bacillus panis (Migula) by Spitzer and Epple (76); Bacillus cereus by Hammer and Hussong (41); Bacillus cereus, Bacillus simplex, and Bacillus megatherium by Kelly (45); Bacillus vulgatus by Morrison and Rettger (53); and Bacillus calidolactis by Hussong and Hammer (44).

In addition to the aerobic, spore-forming bacteria various spore-forming anaerobes, as well as yeasts, micrococci, and streptococci, have been responsible for spoilage in evaporated milk, as shown by Savage and Hunwicke (66), Hunziker (43), and Hammer (37, 39).

Morrison and Rettger (53) noted that evaporated milk supplied a consistently favorable, protective and uniform environment which reduced to a minimum any tendency toward variation in the heat resistance of uniform suspensions of spores heated in it.

METHODS

Sources of organisms. The organisms used in the study were secured from four sources, as follows:

A. Isolated directly from spoiled evaporated milk: cultures 1, 5, 9, 10, 11, and 12.

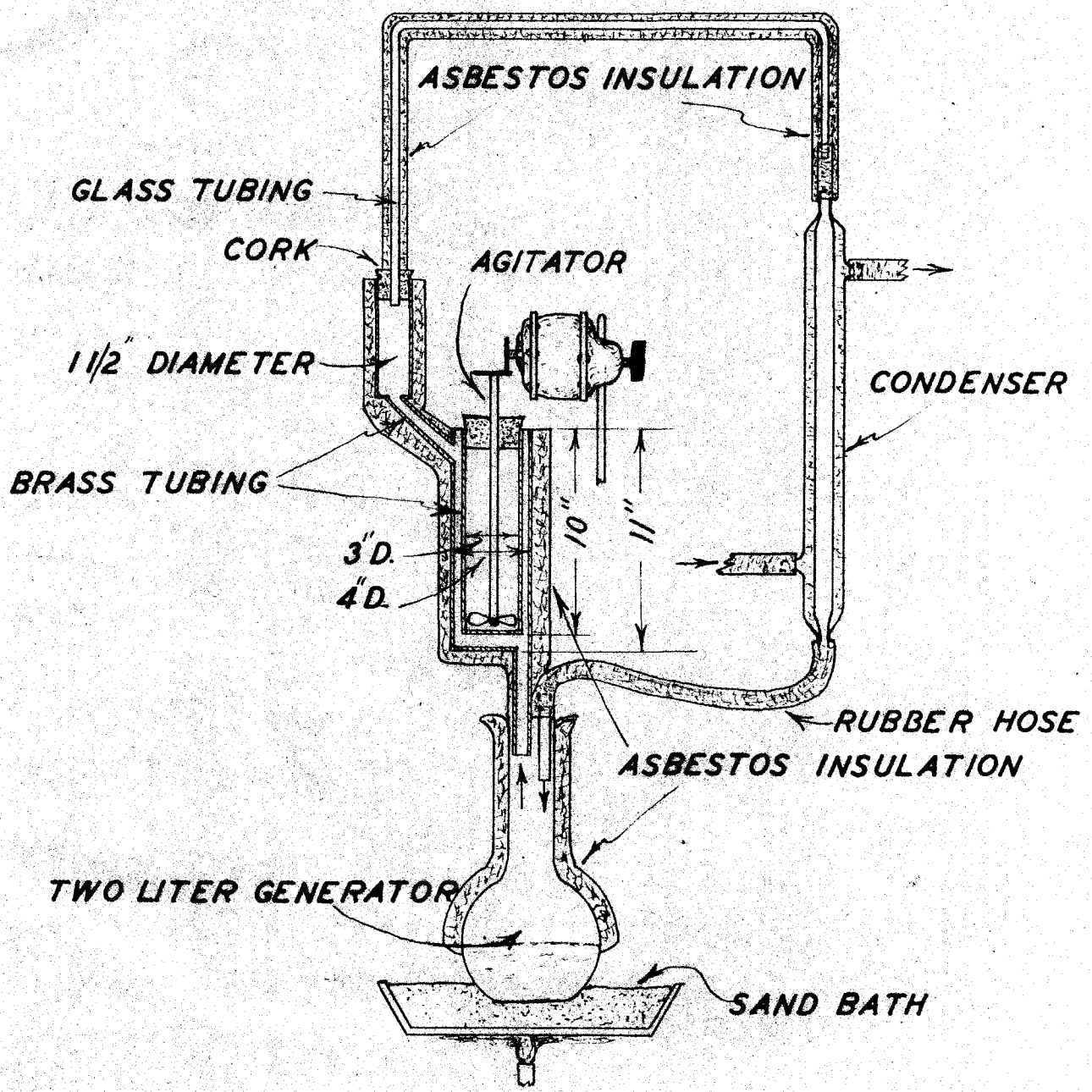
B. Isolated directly from non-spoiled evaporated milk: culture 17.

C. Isolated by a commercial laboratory from non-spoiled evaporated milk: cultures 2, 3, 4, 6, 7, 8, 13, 14, 15, and 16.

D. Isolated from raw milk heated at 80°C. for 10 minutes, enriched at 37° or 55°C., and plated at 21°, 37°, 45°, or 55°C.: cultures A, B, C, D, E, F, G, H, I, J, K, L, and M.

Temperature regulation. Some of the preliminary trials for determining the thermal resistance of the spores of the various cultures were conducted in an insulated, jacketed cylinder, using vapor generated by boiling butyl alcohol as the source of heat. Figure 1 shows the set-up of the equipment as employed in most of the preliminary trials. Approximately one liter of glycerol, kept in motion by a turbine agitator, was used as the heating medium within the cylinder. The cylinder, as originally designed, did not have an outlet of sufficient size to permit the rapid passage of vapor to

APPARATUS USED IN PRELIMINARY TRIALS
FIGURE 1



the condenser but this defect was easily remedied. A coil condenser was used in the first few trials and the temperature controlled very successfully. This condenser was accidentally broken and, although replaced by a similar condenser, difficulty was encountered in controlling temperature fluctuations. Various devices were tried in an attempt to overcome these fluctuations such as a water or mercury column sealed in the side of the delivery arm to the condenser and by closing the entire system. None of the devices tried were entirely satisfactory, but it is believed that further work may prove fruitful. It was planned to use other organic liquids having a boiling point of approximately 116.0°C. but none could be found which were both inexpensive and non-injurious to health, so only redistilled butyl alcohol with a boiling point between 115.0° and 116.0°C. was used as the source of vapor for heating the glycerol.

Most of the preliminary trials and all the major portion of the study were conducted in a DeKhotinsky constant temperature bath equipped with a high speed turbine agitator. Light mineral oil was used as the heating medium. Temperatures were maintained within the limits of ± 0.2°C.

Spore suspension medium. Except for a series of comparative trials with evaporated milk, sterile skimmilk was always used as the spore suspension medium.

Containers in which spore suspensions were heated. Sterile agglutination tubes were used as the containers for the spore suspensions in all thermal resistance trials. The tubes were approximately 10 mm. in outside diameter by 75 mm. long with about 1 mm. thickness of wall.

Amount of spore suspension medium used for heating. In order to reduce the factor of heat penetration to a minimum, small samples were used in all heating trials. The sterilized agglutination tubes were partially filled with 2.0 cc. samples of the spore suspension used and then sealed in a blast lamp flame.

Culture media. Various culture media were used with the different cultures in order to secure the best possible growth and spore production. Nutrient agar proved very satisfactory for growth and development of spores with cultures 1, 2, 3, 4, 7, 8, 9, 13, 15, 16, A, B, C, D, E, F, G, H, I, J, K, L, and M. It was necessary to resort to beef infusion agar for satisfactory growth and development of spores with cultures 5, 10, 11, 12, and 17. Litmus milk was used as the medium to determine sterility with cultures 1 and 5 while with all other cultures dextrose broth containing bromocresol purple was employed. In the identification of the various organisms, media customarily resorted to for this purpose were prepared, such as gelatin, starch agar, potato, the many

sugar broths, etc. All media were made according to methods recommended by the "Manual of Methods for Pure Culture Study of Bacteria" prepared by the Committee on Bacteriological Technic of the Society of American Bacteriologists.

Growth temperatures. Using suitable culture media for the various cultures, as given above, an effort was made to secure growth of each organism at 10°, 21°, 37°, 45°, and 55°C. No growth was secured at 10°C., and none of the organisms had a growth range from 21° to 55°C. The following summary shows the temperatures at which growth was secured with the various cultures.

Growth of various cultures at different temperatures

Culture Number	Growth at 10°C.	21°C.	37°C.	45°C.	55°C.
1	-	+	+	+	-
2	-	+	+	+	-
3	-	+	+	-	-
4	-	+	+	+	-
5	-	-	-	+	+
7	-	+	+	+	-
8	-	+	+	+	-
9	-	-	+	+	+
10	-	-	-	+	+
11	-	-	-	+	+
12	-	-	-	+	+
13	-	+	+	-	-
15	-	+	+	+	-
16	-	+	+	+	-
17	-	+	+	+	-
A	-	-	+	+	+
B	-	-	-	-	+
C	-	-	+	+	+
D	-	-	+	+	-
E	-	+	+	+	-
F	-	-	+	+	+
G	-	-	+	+	+
H	-	-	-	-	+
I	-	-	-	-	+
J	-	-	-	-	+
K	-	-	-	-	+
L	-	-	-	-	+
M	-	-	-	-	+

Preparation of spore suspensions. Nutrient or beef infusion agar slants were inoculated with a pure culture of the organism to be tested and grown at one of the temperatures which preliminary work indicated would permit growth and development of spores.

The periods of incubation at the different growth temperatures were varied in order to permit as large a production of spores as possible and yet secure comparisons of spores of approximately the same age. The production of spores was slower at low growth temperatures than at higher temperatures which necessitated a longer period of incubation at the low temperatures in order to secure approximately the same spore development as at the higher temperatures. The maximum periods of incubation at the higher temperatures were in turn limited by the injurious effect of the higher temperatures on the spores. Long periods of incubation at high temperatures decreased the number of viable spores on the agar slants. The incubation periods were as follows:

A. 21°C. - 5 days

B. 37°C. - 5 days

C. 45°C. - 3 days

D. 55°C. - 3 days

A portion of each growth was transferred to 60 cc. of sterile skimmilk and the skimmilk then agitated vigorously for several minutes. The spore content per cubic centimeter

of skimmilk in comparative thermal resistance trials was relatively constant due to the care with which definite quantities of growth were transferred. Thus the growth temperature was the only variable factor. Each of eight sterilized agglutination tubes was then partially filled with 2.0 cc. of the spore suspension and sealed in a blast lamp flame. The tubes were immediately heated in the oil bath and the time between removal of the spores from the incubator and immersion in the oil bath never exceeded 20 minutes. Although eight tubes were prepared for each trial, only seven exposures were used and the eighth tube was needed only when a tube was accidentally broken.

The skimmilk remaining after filling the agglutination tubes was heated at 80°C. for 10 minutes, and plated for spore content on the assumption that the spores but not the vegetative cells would survive this exposure.

Preparation of spore powder. In the preparation of spore powder nutrient broth cultures were used to inoculate the surface of nutrient or beef infusion agar in a series of petri dishes. The dishes were incubated at 21°, 37°, or 45°C. for 5 days or at 55°C. for 3 days. At the end of the incubation period the surface growth was scraped into a sterile petri dish and dried over calcium chloride in a partial vacuum at 37°C. for 48 hours. The material was then ground in a sterile mortar and well mixed with sterile powdered lactose.

All spore powders were kept in sterile glass-stoppered two-ounce bottles, at room temperature, over calcium chloride in a desiccator.

The spore content of the powder was determined by adding approximately 0.01 gram to 100 cc. of sterile water and plating after holding at 80°C. for 10 minutes. This small portion of spore powder was measured out by means of a sterile measuring spoon having approximately 0.01 gram capacity. Each measure of powder was filled and scraped off in the same manner in order to assure equivalent portions. Repeated weighings demonstrated that approximately 0.01 gram portions of spore powder were regularly secured by this method.

In testing the heat resistance of the spores in a powder, the powder was transferred directly to 60 cc. of sterile skimmilk by means of a sterile measuring spoon (0.01 gram capacity). The amount of powder was varied in accordance with the spore content of the powder. After the transfer to skimmilk, the procedure for preparing the spore suspensions was the same as when using direct transfers of spores from agar slants.

In each trial on the comparative heat resistance of moist and dry spores or dry spores of different ages, every effort was made to secure spore suspensions containing approximately the same number of spores per cubic centimeter. In

some instances it was necessary to make several runs before satisfactory spore counts were secured in comparative trials. By this means the only variable factor influencing heat resistance was the growth temperature.

Exposure temperatures. The spores were exposed to heat by immersing the sealed tubes containing the spore suspension in the oil bath. Before the tubes were subjected to the desired temperature they were exposed to the same temperature for 15 seconds in a preliminary oil bath. The exposure temperatures used, except for slight variations with specific cultures, were:

A. 104.0°C.

B. 108.0°C.

C. 112.0°C.

D. 116.0°C.

E. 120.0°C.

The series of eight agglutination tubes, each containing 2.0 cc. of the spore suspension, was exposed to the desired temperature for definite periods of time.

With each culture the exposure periods used in the preliminary trial were 3, 5, 7, 10, 15, 20, and 25 minutes. After the first trial, the exposure periods were usually varied in order to have about the same number of periods above and below the period last showing growth. For example, if the preliminary trial showed the last survival at 7 minutes the

exposure periods were then changed to 3, 4, 5, 7, 9, 12, and 15 minutes. In practically all trials other than the preliminary ones the differences between exposures were 1 minute from 0 to 5 minutes, 2 minutes from 5 to 9 minutes, 3 minutes from 9 to 15 minutes, and 5 minutes from 15 to 40. At the end of each exposure period a tube was removed, immediately placed in a bath of cold water, and tested for sterility within 10 minutes.

Determination of sterility. Sterility of a spore suspension after exposure to heat was determined by inoculating a tube of litmus milk or dextrose broth containing bromocresol purple with a 1 cc. portion of the contents of the heated tube and incubating for 7 days at the optimum growth temperature for the culture. As a check, several loops of the heated spore suspension were streaked on a nutrient or beef infusion agar slant, and the slant incubated at the optimum growth temperature for 7 days.

Determination of pH. All hydrogen-ion determinations were made electrometrically, using quinhydrone, and calculated to the nearest 0.1.

RESULTS

Comparison of heat resistance of spores suspended in skimmilk and in evaporated milk

Various investigators, notably Ayers and Johnson (2), Barthel and Stenstrom (3), and Brown and Peiser (10), have demonstrated that milk, when used as the suspension medium for the vegetative cells or spores of bacteria, aids them in resisting destruction by heat. Therefore, in determining the thermal resistance of any organism in milk, due consideration should be given to the influence of the protective action of the kind of milk used.

In studying the influence of growth temperature on the thermal resistance of some aerobic, spore-forming bacteria from evaporated milk it was planned to use either sterilized skimmilk or evaporated milk as the suspension medium instead of sterile water or broth, so that the conditions of heating would be more nearly comparable to those existing during the process of sterilizing evaporated milk. In order to determine the difference, if any, between the protective action of sterilized skimmilk and evaporated milk, a series of comparative trials was run with five representative cultures.

Every effort was made to have the conditions as nearly identical as possible except for the type of suspen-

sion medium. In each comparison, the spore content per cubic centimeter of skimmilk and evaporated milk varied only a little, and since the spores were from one source the growth temperature and age were the same. The evaporated milk used was not resterilized but was transferred aseptically from commercial cans of evaporated milk. It had a pH of 6.5 while the pH of the skimmilk was 6.4.

Results of the trials are presented in Table 1. In 3 of the 5 comparisons there was no difference in the protective action of the two suspension media. With cultures 2 and 4, however, the skimmilk apparently had a slightly greater protective action than did the evaporated milk. In each instance the difference was only two minutes at the temperature used and does not appear to be significant, especially since the results of the three other comparisons showed no difference between the two media.

From the data presented it appears that results of thermal resistance trials using sterilized skimmilk as the spore suspension medium are comparable to the results secured with evaporated milk as the suspension medium.

When it was found that there was little difference between the protective action of skimmilk and of evaporated milk, it was decided to use skimmilk as the suspension medium for the spores in the thermal resistance trials. The decision was also based upon two other reasons: first, evaporated milk

Table 1

Comparison of heat resistance of spores suspended in skimmilk and in evaporated milk (Temperature of exposure 116.0°C.)

Culture used to secure spores			Milk used for suspension	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
No.	Growth temperature °C.	Age (days)				
1	37	5	skim	17,400	2,3,4,5,7,9,11	7.0
			evap.	18,000	2,3,4,5,7,9,11	7.0
2	37	5	skim	3,500	16,18,20,22,24,26,28	20.0
			evap.	4,500	16,18,20,22,24,26,28	18.0
4	37	5	skim	250,000	16,18,20,22,24,26,28	20.0
			evap.	290,000	16,18,20,22,24,26,28	18.0
5	55	3	skim	450	26,28,30,32,34,36,38	30.0
			evap.	500	26,28,30,32,34,36,38	30.0
9	45	3	skim	40,000	1,2,3,4,5,7,9	2.0
			evap.	45,000	1,2,3,4,5,7,9	2.0

is difficult to handle in large quantities without contamination; and second, it was considered inadvisable to resterilize evaporated milk since there is a possibility of changing its composition and consequently modifying its protective action on the suspended spores.

Comparative thermal resistance of spores of various bacteria grown at two different temperatures

In an effort to secure information on the influence of growth temperature on the thermal resistance of bacterial spores, all of the cultures examined which showed any appreciable resistance to heat were grown at two different temperatures and the spores tested for heat resistance. The higher of the two growth temperatures was, in all instances, the optimum or at least the temperature among those used which gave the most luxuriant growth of the culture being studied. The optimum growth temperatures employed were 37°, 45°, and 55°C., while the lower growth temperatures used in comparison with these temperatures were 21°, 37°, and 45°C., respectively. Before being used in comparative trials, each culture was carried through at least three transfers at the respective growth temperatures.

The results of the comparative trials with the various cultures are presented in Table 2. An analysis of the data shows that in 53 out of 57 trials the spores possessed

Table 2

Comparative thermal resistance of spores of various bacteria grown at two different temperatures

Trial No.	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)
Culture 1, 5 days old, heated at 114.5°C. pH of milk 6.3				
1	21	149,000	3,5,7,10,15,20,25	5.0
	37	24,000	3,5,7,10,15,20,25	15.0
2	21	9,500	2,3,4,5,7,9,11	3.0
	37	7,000	7,9,12,15,20,25,30	12.0
3	21	39,000	2,3,4,5,7,9,11	7.0
	37	75,000	7,9,12,15,20,25,30	12.0
4	21	240,000	2,3,4,5,7,9,11	5.0
	37	130,000	7,9,12,15,20,25,30	12.0
5	21	12,000	2,3,4,5,7,9,11	5.0
	37	45,000	7,9,12,15,20,25,30	20.0
6	21	67,000	2,3,4,5,7,9,11	3.0
	37	77,000	7,9,12,15,20,25,30	20.0
7	21	32,000	2,3,4,5,7,9,11	5.0
	37	40,000	7,9,12,15,20,25,30	20.0
8	21	51,500	2,3,4,5,7,9,11	5.0
	37	76,000	7,9,12,15,20,25,30	25.0
9	21	120,000	2,3,4,5,7,9,11	5.0
	37	57,000	7,9,12,15,20,25,30	15.0
10	21	170,000	2,3,4,5,7,9,11	5.0
	37	94,000	7,9,12,15,20,25,30	20.0
11	21	145,000	2,3,4,5,7,9,11	3.0
	37	68,000	7,9,12,15,20,25,30	15.0

Table 2 (Cont.)

Trial No.	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)
Culture 2, 5 days old, heated at 116.0°C. pH of milk 6.3				
1	21	35,500	3,5,7,10,15,20,25	7.0
	37	53,000	3,5,7,10,15,20,25	15.0
2	21	30,000	3,4,5,7,9,12,15	9.0
	37	66,000	7,9,12,15,20,25,30	25.0
3	21	55,000	3,4,5,7,9,12,15	9.0
	37	43,500	7,9,12,15,20,25,30	25.0
4	21	26,500	3,4,5,7,9,12,15	9.0
	37	59,500	7,9,12,15,20,25,30	25.0
5	21	21,000	3,4,5,7,9,12,15	7.0
	37	100,000	7,9,12,15,20,25,30	25.0
6	21	100,000	3,4,5,7,9,12,15	9.0
	37	35,000	7,9,12,15,20,25,30	15.0
7	21	10,500	3,4,5,7,9,12,15	12.0
	37	10,000	7,9,12,15,20,25,30	20.0
8	21	26,000	3,4,5,7,9,12,15	9.0
	37	30,000	7,9,12,15,20,25,30	25.0
9	21	16,000	3,4,5,7,9,12,15	9.0
	37	13,500	7,9,12,15,20,25,30	15.0
10	21	13,000	3,4,5,7,9,12,15	5.0
	37	11,000	7,9,12,15,20,25,30	9.0
Culture 3, 5 days old, heated at 116.0°C. pH of milk 6.4				
1	21	1,200,000	3,5,7,10,15,20,25	None
	37	1,500,000	3,5,7,10,15,20,25	3.0
2	21	5,000,000	1,2,3,4,5,7,9	None
	37	4,500,000	1,2,3,4,5,7,9	3.0

Table 2 (Cont.)

Trial No.	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)
Culture 3, 5 days old, heated at 116.0°C. pH of milk 6.4				
3	21	4,300,000	1,2,3,4,5,7,9	3.0
	37	5,500,000	1,2,3,4,5,7,9	3.0
4	21	1,300,000	1,2,3,4,5,7,9	None
	37	1,100,000	1,2,3,4,5,7,9	3.0
5	21	1,600,000	1,2,3,4,5,7,9	None
	37	5,400,000	1,2,3,4,5,7,9	3.0
Culture 4, 5 days old, heated at 116.0°C. pH of milk 6.3				
1	21	16,000	3,5,7,10,15,20,25	5.0
	37	27,000	3,5,7,10,15,20,25	10.0
2	21	16,000	3,4,5,7,9,12,15	7.0
	37	15,000	5,7,9,12,15,20,25	20.0
3	21	18,000	3,4,5,7,9,12,15	9.0
	37	22,000	5,7,9,12,15,20,25	25.0
4	21	18,000	3,4,5,7,9,12,15	7.0
	37	51,000	5,7,9,12,15,20,25	20.0
5	21	16,000	3,4,5,7,9,12,15	5.0
	37	10,000	5,7,9,12,15,20,25	25.0
6	21	14,000	3,4,5,7,9,12,15	5.0
	37	12,000	5,7,9,12,15,20,25	15.0
7	21	18,000	3,4,5,7,9,12,15	7.0
	37	13,000	5,7,9,12,15,20,25	20.0
8	21	11,000	3,4,5,7,9,12,15	5.0
	37	16,000	5,7,9,12,15,20,25	15.0

Table 2 (Cont.)

Trial No.	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)
Culture 5, 3 days old, heated at 120.0°C. pH of milk 6.4				
1	45	500	3,5,7,10,15,20,25	5.0
	55	800	3,5,7,10,15,20,25	20.0
2	45	1,000	3,4,5,7,9,12,15	7.0
	55	2,000	9,12,15,20,25,30,35	25.0
3	45	5,000	3,4,5,7,9,12,15	9.0
	55	10,050	9,12,15,20,25,30,35	25.0
4	45	1,000	3,4,5,7,9,12,15	7.0
	55	7,600	9,12,15,20,25,30,35	25.0
5	45	200	3,4,5,7,9,12,15	5.0
	55	100	9,12,15,20,25,30,35	25.0
6	45	100	3,4,5,7,9,12,15	5.0
	55	200	9,12,15,20,25,30,35	25.0
7	45	100	3,4,5,7,9,12,15	5.0
	55	250	9,12,15,20,25,30,35	20.0
Culture 7, 5 days old, heated at 106.0°C. pH of milk 6.3				
1	21	40,000	3,5,7,10,15,20,25	3.0
	37	70,000	3,5,7,10,15,20,25	7.0
2	21	1,000,000	1,2,3,4,5,7,9	3.0
	37	800,000	3,4,5,7,9,12,15	7.0
Culture 8, 5 days old, heated at 106.0°C. pH of milk 6.3				
1	21	450,000	3,5,7,10,15,20,25	None
	37	1,000,000	3,5,7,10,15,20,25	3.0
2	21	800,000	1,2,3,4,5,7,9	None
	37	440,000	1,2,3,4,5,7,9	3.0

Table 2 (Cont.)

Trial No.	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)
Culture 9, 5 days old, heated at 110.0°C. pH of milk 6.3				
1	37	80,000	3,5,7,10,15,20,25	3.0
	45	60,000	3,5,7,10,15,20,25	5.0
2	37	100,000	1,2,3,4,5,7,9	5.0
	45	80,000	2,3,4,5,7,9,12	5.0
3	37	200,000	1,2,3,4,5,7,9	5.0
	45	170,000	2,3,4,5,7,9,12	5.0
4	37	250,000	1,2,3,4,5,7,9	3.0
	45	120,000	2,3,4,5,7,9,12	5.0
5	37	210,000	1,2,3,4,5,7,9	5.0
	45	170,000	2,3,4,5,7,9,12	7.0
6	37	300,000	1,2,3,4,5,7,9	5.0
	45	280,000	2,3,4,5,7,9,12	5.0
Culture 10, 3 days old, heated at 116.0°C. pH of milk 6.3				
1	45	600	3,5,7,10,15,20,25	10.0
	55	800	15,20,25,30,35,40,45	30.0
2	45	410	3,5,7,10,15,20,25	10.0
	55	500	15,20,25,30,35,40,45	30.0
Culture 11, 3 days old, heated at 116.0°C. pH of milk 6.3				
1	45	11,700	15,20,25,30,35,40,45	25.0
	55	17,800	15,20,25,30,35,40,45	30.0
2	45	11,300	15,20,25,30,35,40,45	25.0
	55	3,000	15,20,25,30,35,40,45	35.0

Table 2 (Cont.)

Trial No.	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)
Culture 12, 3 days old, heated at 116.0°C. pH of milk 6.3				
1	45	12,300	5,7,9,12,15,20,25	5.0
	55	11,400	15,20,25,30,35,40,45	30.0
2	45	9,000	5,7,9,12,15,20,25	15.0
	55	8,000	15,20,25,30,35,40,45	30.0

a greater thermal resistance when grown at the optimum growth temperature. Trial 3 with culture 3 and trials 2, 3, and 6 with culture 9 showed no difference in the thermal resistance of the spores grown at the two different temperatures. With culture 3, however, trials 1, 2, 4, and 5 (or 4 out of 5 trials) showed a greater resistance for the spores grown at the optimum temperature, as was also the case in trials 1, 4, and 5 with culture 9. It is well to note that there was no instance of spores from a culture grown at a temperature below the optimum exhibiting a thermal resistance greater than that of spores from a culture grown at the optimum temperature.

The spore content, per cubic centimeter of skimmilk, varied somewhat in the comparative trials but no significant difference in thermal resistance could be attached to any definite difference in the spore content. Within the limits of the spore contents used in the individual trials, there did not appear to be any correlation between the number of spores present per cubic centimeter of skimmilk and the time of survival of the spores.

The data in Table 2 are summarized in Table 3. The summary shows that on the average the spore content per cubic centimeter of skimmilk in comparative trials varied but little. It also shows that every culture possessed a greater average thermal resistance when grown at the optimum growth temperature than when grown below the optimum. The greater

Table 3

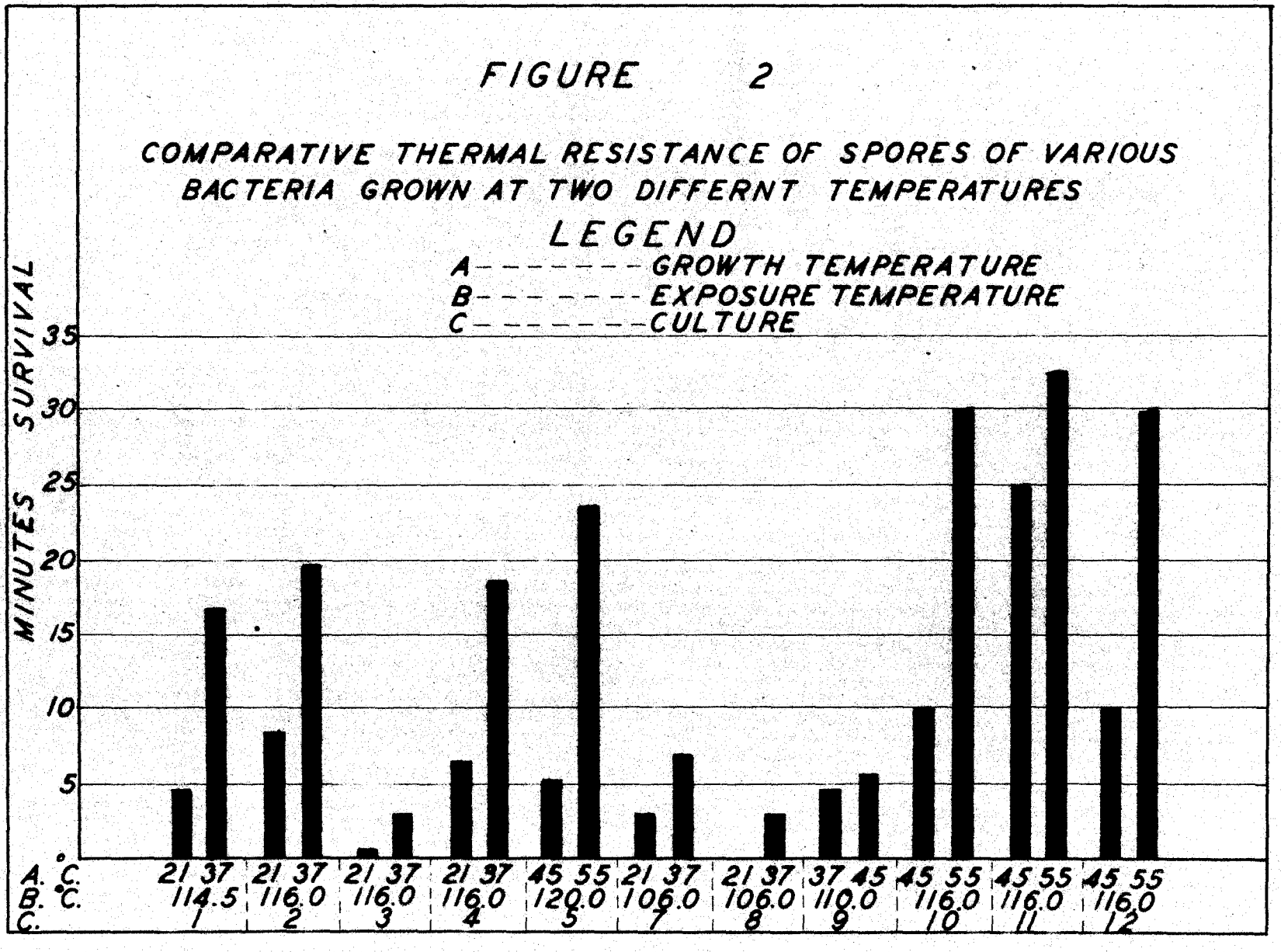
Comparative thermal resistance of spores of various bacteria grown at two different temperatures (Summary of data in Table 2)

Culture	No. of trials	Growth temperature °C.	Age of culture (days)	Exposure temperature °C.	Spores per cc. of milk heated (average)	Survival (in min.) (average)	Range of survival (minutes)
1	11	21	5	114.5	71,363	4.6	3 - 7
1	11	37	5	114.5	63,000	16.9	12 - 25
2	10	21	5	116.0	33,350	8.5	7 - 15
2	10	37	5	116.0	42,150	19.9	9 - 25
3	5	21	5	116.0	2,680,000	0.6	0 - 3
3	5	37	5	116.0	3,600,000	3.0	3 - 3
4	8	21	5	116.0	15,870	6.3	5 - 9
4	8	37	5	116.0	20,750	18.8	10 - 25
5	7	45	3	120.0	1,128	6.1	5 - 9
5	7	55	3	120.0	3,000	23.6	20 - 25
7	2	21	5	106.0	520,000	3.0	3 - 3
7	2	37	5	106.0	435,000	7.0	7 - 7
8	2	21	5	106.0	675,000	0.0	0 - 0
8	2	37	5	106.0	720,000	3.0	3 - 3
9	6	37	5	110.0	190,000	4.3	3 - 5
9	6	45	5	110.0	146,000	5.3	5 - 7
10	2	45	3	116.0	505	10.0	10 - 10
10	2	55	3	116.0	650	30.0	30 - 30
11	2	45	3	116.0	11,500	25.0	25 - 25
11	2	55	3	116.0	10,400	32.5	30 - 35
12	2	45	3	116.0	10,650	10.0	5 - 15
12	2	55	3	116.0	9,700	30.0	30 - 30

thermal resistance exhibited at the optimum growth temperature was, except for culture 9, very significant. With culture 9 the greater thermal resistance caused by growth at its optimum growth temperature was comparatively small, being only one minute. The data definitely demonstrate that, with the cultures studied, growth at a temperature below the optimum decreased the thermal resistance. The differences in average thermal resistance of the spores of the various cultures when grown at the two temperatures are clearly shown in Figure 2. The increases in thermal resistance of the cultures when grown at the optimum growth temperatures were particularly striking with cultures 1, 2, 4, 5, 10, 11, and 12, being 12.3, 11.4, 12.5, 17.5, 20.0, 7.5, and 20.0 minutes, respectively. These cultures were exposed to temperatures approximating the sterilization temperature used for evaporated milk. Culture 1 was exposed to 114.5°C., cultures 2, 4, 10, 11, and 12 to 116.0°C., and culture 5 to 120.0°C. At these temperatures the spores, when grown at the optimum growth temperature, survived longer than the normal holding period used in sterilizing evaporated milk which is from 15 to 17 minutes. The average survival was 16.9, 19.9, 18.8, 23.6, 30.0, 32.5, and 30.0 minutes for cultures 1, 2, 4, 5, 10, 11, and 12, respectively. Outstanding in resistance to heat was culture 5 which, when grown at 55°C., survived a temperature of 120.0°C. for 23.6 minutes. This thermal resistance is sufficient to

FIGURE 2

COMPARATIVE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA GROWN AT TWO DIFFERNT TEMPERATURES



enable the organism to survive the ordinary autoclaving procedure used in laboratory work which demands a temperature of 120.0°C. for 20 minutes.

The observations reported in Tables 2 and 3 indicate that spores of some of the organisms found in evaporated milk can survive, when grown at their optimum temperature and present in large numbers, the sterilization process normally used in the manufacture of evaporated milk.

Comparative thermal resistance of spores of various bacteria grown at three different temperatures

Having established the fact that a growth temperature below the optimum lowered the thermal resistance of the spores of the cultures studied, it was thought advisable to try other growth temperatures. Cultures 2, 4, 7, 8, and 9 were the only cultures which, with the temperatures used, showed growth at a temperature above the optimum and also at a temperature below the optimum. These cultures were carried through at least three transfers at the respective growth temperatures before being used in comparative thermal resistance trials. The pH of the skimmilk was 6.3 in all cases.

Table 4 presents the results of the comparative trials with three growth temperatures. In 9 out of 10 trials, spores produced by cultures grown at the optimum temperature exhibited a greater thermal resistance than spores

Table 4

Comparative thermal resistance of spores of various bacteria grown at three different temperatures

Trial No.	Growth temperature °C.	Age of culture (days)	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 2, heated at 116.0°C. pH of milk 6.3					
1	21	5	13,000	3,4,5,7,9,12,15	3.0
	37	5	11,000	7,9,12,15,20,25,30	9.0
	45	3	300	3,4,5,7,9,12,15	3.0
2	21	5	2,000	3,4,5,7,9,12,15	3.0
	37	5	1,500	7,9,12,15,20,25,30	15.0
	45	3	2,300	3,4,5,7,9,12,15	7.0
Culture 4, heated at 116.0°C. pH of milk 6.3					
1	21	5	163,000	3,4,5,7,9,12,15	3.0
	37	5	171,000	5,7,9,12,15,20,25	15.0
	45	3	11,000	3,4,5,7,9,12,15	3.0
2	21	5	125,000	3,4,5,7,9,12,15	9.0
	37	5	100,000	5,7,9,12,15,20,25	20.0
	45	3	40,000	3,4,5,7,9,12,15	9.0
Culture 7, heated at 106.0°C. pH of milk 6.3					
1	21	5	1,300,000	1,2,3,4,5,7,9	3.0
	37	5	700,000	3,4,5,7,9,12,15	7.0
	45	3	1,800,000	1,2,3,4,5,7,9	7.0
2	21	5	2,100,000	1,2,3,4,5,7,9	3.0
	37	5	1,300,000	3,4,5,7,9,12,15	7.0
	45	3	1,200,000	1,2,3,4,5,7,9	7.0

Table 4 (Cont.)

Trial No.	Growth temperature °C.	Age of culture (days)	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 8, heated at 106.0°C. pH of milk 6.3					
1	21	5	1,400,000	1,2,3,4,5,7,9	None
	37	5	1,300,000	1,2,3,4,5,7,9	3.0
	45	5	800,000	1,2,3,4,5,7,9	None
2	21	5	400,000	1,2,3,4,5,7,9	None
	37	5	700,000	1,2,3,4,5,7,9	3.0
	45	3	400,000	1,2,3,4,5,7,9	None
Culture 9, heated at 110.0°C. pH of milk 6.3					
1	37	5	300,000	1,2,3,4,5,7,9	3.0
	45	3	280,000	2,3,4,5,7,9,12	5.0
	55	3	76,000	1,2,3,4,5,7,9	3.0
2	37	5	31,000	1,2,3,4,5,7,9	5.0
	45	3	10,000	2,3,4,5,7,9,12	5.0
	55	3	10,000	1,2,3,4,5,7,9	5.0

from cultures grown at temperatures below the optimum. The single exception to this trend was trial 2 with culture 9 in which the heat resistance was the same for spores from cultures grown at the three temperatures. With 7 out of 10 trials, spores from cultures grown at the optimum temperature possessed a greater thermal resistance than spores from cultures grown at temperatures above the optimum. Trials 1 and 2 with culture 7 and trial 2 with culture 9 showed the same heat resistance for spores from cultures grown at a temperature above the optimum as for spores from cultures grown at the optimum. The data also show that in 7 out of 10 trials, the spores from cultures grown at the optimum temperature exhibited greater resistance to heat than spores from cultures grown below or above the optimum. The spore content per cubic centimeter of skimmilk varied in individual trials, but apparently this variation was in no way related to the length of time the spores survived the heat treatment.

The average spore content per cubic centimeter of skimmilk and the average survival of the spores of the various cultures are shown in Table 5. The average values for survival show about the same differences in heat resistance for the various cultures as do the individual trials. Four of the 5 cultures, namely, cultures 2, 4, 8, and 9, showed their maximum thermal resistance when grown at their optimum

Table 5

Comparative thermal resistance of spores of various bacteria grown at three different temperatures (Summary of data in Table 4)

Culture	No. of trials	Growth temperature °C.	Age of culture (days)	Exposure temperature °C.	Spores per cc. of milk heated (average)	Survival (in minutes) (average)
2	2	21	5	116.0	7,500	3.0
2	2	37	5	116.0	6,250	12.0
2	2	45	3	116.0	1,300	5.0
4	2	21	5	116.0	144,000	6.0
4	2	37	5	116.0	135,500	17.5
4	2	45	3	116.0	25,500	6.0
7	2	21	5	106.0	1,700,000	3.0
7	2	37	5	106.0	1,000,000	7.0
7	2	45	3	106.0	1,500,000	7.0
8	2	21	5	106.0	900,000	0.0
8	2	37	5	106.0	1,000,000	3.0
8	2	45	3	106.0	600,000	0.0
9	2	37	5	110.0	160,500	4.0
9	2	45	3	110.0	145,000	5.0
9	2	55	5	110.0	43,000	4.0

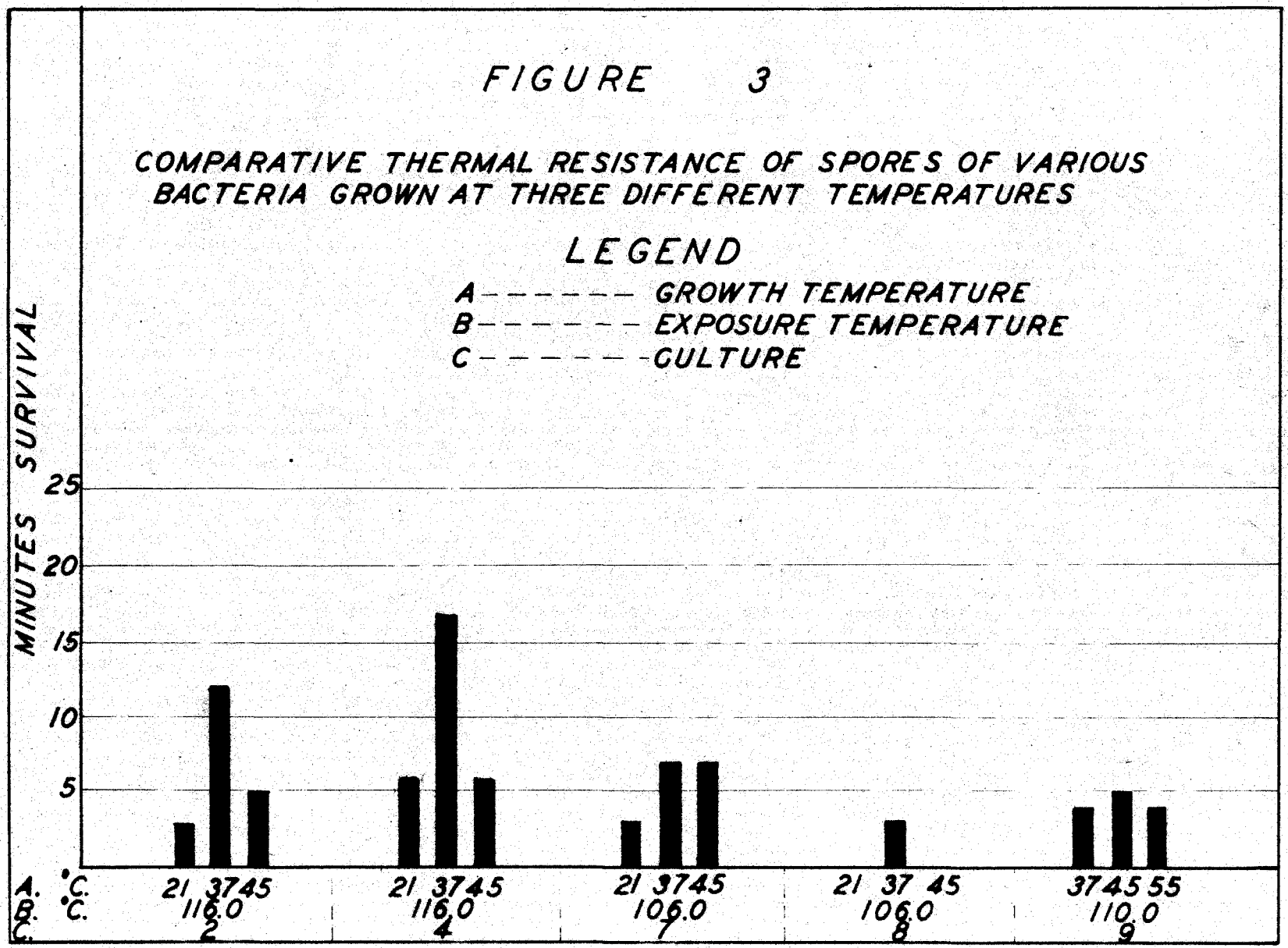
growth temperature. Culture 7, however, exhibited just as great a resistance to heat when grown above the optimum as when grown at the optimum temperature. With cultures 2, 4, and 8 the greater thermal resistance exhibited when grown at the optimum growth temperature was quite marked, but with culture 9 the difference was small. There appeared to be little difference, except with culture 7, in the extent of the decrease in thermal resistance caused by growth below the optimum and by growth above the optimum temperature.

Figure 3 clearly shows that the thermal resistance of the spores of the cultures employed was usually lowered when growth temperatures either above or below the optimum were used. In other words, unfavorable growth temperatures decreased the thermal resistance. These results substantiate those secured when two growth temperatures were studied.

If the cultures investigated are representative of those found in evaporated milk, it appears that a low resistance to heat can be effectively secured by maintaining low growth temperatures. From the data secured temperatures of 21°C. or below would be considered as low temperatures. With all the cultures studied a temperature of 21°C. or below resulted either in no growth or in a low thermal resistance of the spores formed.

FIGURE 3

COMPARATIVE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA GROWN AT THREE DIFFERENT TEMPERATURES



Influence of a sudden change in growth temperature on the thermal resistance of spores of various bacteria

In all the trials thus far reported, each culture was carried through a series of at least three transfers or generations at a certain temperature before it was used in the thermal resistance trials. Speculation as to the influence of a sudden change in the growth temperature naturally arose and a series of trials was planned in an effort to evaluate this factor.

After cultures 1, 2, 3, 4, 5, and 9 had been growing for some time at two different temperatures, of which the higher temperature was the optimum temperature, the growth temperatures of the cultures were suddenly changed. The same growth temperatures were used for each culture as formerly but the transfers of the cultures were held at a temperature different than the one used with the cultures from which the transfers came. For example, if a transfer was made from a culture growing at 37°C. (the optimum temperature for the culture) the transfer was grown at 21°C., while if the transfer was made from a culture of the same organism growing at 21°C, it was grown at 37°C.

Table 6 presents the results of the thermal resistance trials on spores of cultures whose growth temperatures had been suddenly changed. In 19 out of 21 trials a sudden change of growth temperature from below the optimum to the

Table 6

Influence of a sudden change in growth temperature on the thermal resistance of spores of various bacteria

Trial No.	Culture	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 1, 5 days old, heated at 114.5°C. pH of milk 6.3					
1	1	21	160,000	3,5,7,10,15,20,25	7.0
	1-A	37	200,000	3,5,7,10,15,20,25	10.0
	1-B	21	260,000	3,5,7,10,15,20,25	10.0
	1	37	120,000	3,5,7,10,15,20,25	20.0
2	1	21	192,000	2,3,4,5,7,9,12	5.0
	1-A	37	15,000	2,3,4,5,7,9,12	3.0
	1-B	21	300,000	2,3,4,5,7,9,12	7.0
	1	37	130,000	7,9,12,15,20,25,30	15.0
3	1	21	280,000	2,3,4,5,7,9,12	3.0
	1-A	37	14,500	2,3,4,5,7,9,12	5.0
	1-B	21	320,000	2,3,4,5,7,9,12	7.0
	1	37	160,000	7,9,12,15,20,25,30	15.0
4	1	21	22,500	2,3,4,5,7,9,12	3.0
	1-A	37	88,000	2,3,4,5,7,9,12	5.0
	1-B	21	400,000	2,3,4,5,7,9,12	5.0
	1	37	240,000	7,9,12,15,20,25,30	25.0
Culture 2, 5 days old, heated at 116.0°C. pH of milk 6.3					
1	2	21	100,000	3,5,7,10,15,20,25	7.0
	2-A	37	200,000	3,5,7,10,15,20,25	10.0
	2-B	21	40,000	3,5,7,10,15,20,25	10.0
	2	37	35,000	3,5,7,10,15,20,25	20.0
2	2	21	10,500	3,4,5,7,9,12,15	7.0
	2-A	37	200,000	7,9,12,15,20,25,30	20.0
	2-B	21	50,000	3,4,5,7,9,12,15	7.0
	2	37	10,000	7,9,12,15,20,25,30	15.0

Table 6 (Cont.)

Trial No.	Culture	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 2, 5 days old, heated at 116.0°C. pH of milk 6.3					
3	2	21	26,000	3,4,5,7,9,12,15	7.0
	2-A	37	100,000	7,9,12,15,20,25,30	15.0
	2-B	21	22,000	3,4,5,7,9,12,15	5.0
	2	37	16,000	7,9,12,15,20,25,30	15.0
4	2	21	30,000	3,4,5,7,9,12,15	5.0
	2-A	37	150,000	7,9,12,15,20,25,30	20.0
	2-B	21	10,000	3,4,5,7,9,12,15	3.0
	2	37	35,000	7,9,12,15,20,25,30	15.0
Culture 3, 5 days old, heated at 110.0°C. pH of milk 6.4					
1	3	21	2,000,000	1,2,3,4,5,7,9	None
	3-A	37	3,000,000	1,2,3,4,5,7,9	5.0
	3-B	21	1,000,000	1,2,3,4,5,7,9	None
	3	37	2,000,000	1,2,3,4,5,7,9	10.0
2	3	21	1,600,000	1,2,3,4,5,7,9	None
	3-A	37	3,300,000	1,2,3,4,5,7,9	3.0
	3-B	21	1,200,000	1,2,3,4,5,7,9	3.0
	3	37	1,600,000	1,2,3,4,5,7,9	5.0
3	3	21	2,100,000	1,2,3,4,5,7,9	None
	3-A	37	2,500,000	1,2,3,4,5,7,9	5.0
	3-B	21	1,800,000	1,2,3,4,5,7,9	3.0
	3	37	2,800,000	1,2,3,4,5,7,9	5.0
Culture 4, 5 days old, heated at 116.0°C. pH of milk 6.3					
1	4	21	15,000	3,5,7,10,15,20,25	7.0
	4-A	37	18,000	3,5,7,10,15,20,25	10.0
	4-B	21	15,000	3,5,7,10,15,20,25	7.0
	4	37	15,000	3,5,7,10,15,20,25	20.0
2	4	21	16,000	3,5,7,10,15,20,25	5.0
	4-A	37	8,000	9,12,15,20,25,30,35	15.0
	4-B	21	22,000	3,5,7,10,15,20,25	15.0
	4	37	27,000	9,12,15,20,25,30,35	20.0

Table 6 (Cont.)

Trial No.	Culture	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 4, 5 days old, heated at 116.0°C. pH of milk 6.3					
3	4	21	15,000	3,5,7,10,15,20,25	5.0
	4-A	37	5,000	9,12,15,20,25,30,35	15.0
	4-B	21	10,000	3,5,7,10,15,20,25	7.0
	4	37	3,000	9,12,15,20,25,30,35	15.0
4	4	21	4,000	3,5,7,10,15,20,25	7.0
	4-A	37	5,000	9,12,15,20,25,30,35	15.0
	4-B	21	9,000	3,5,7,10,15,20,25	10.0
	4	37	30,000	9,12,15,20,25,30,35	15.0
5	4	21	6,000	3,5,7,10,15,20,25	5.0
	4-A	37	9,000	9,12,15,20,25,30,35	15.0
	4-B	21	4,000	3,5,7,10,15,20,25	10.0
	4	37	4,000	9,12,15,20,25,30,35	25.0
Culture 5, 3 days old, heated at 120.0°C. pH of milk 6.4					
1	5	45	200	3,4,5,7,9,12,15	5.0
	5-A	55	300	9,12,15,20,25,30,35	20.0
	5-B	45	600	3,4,5,7,9,12,15	5.0
	5	55	200	9,12,15,20,25,30,35	25.0
2	5	45	110	3,4,5,7,9,12,15	5.0
	5-A	55	130	9,12,15,20,25,30,35	20.0
	5-B	45	100	3,4,5,7,9,12,15	5.0
	5	55	200	9,12,15,20,25,30,35	25.0
3	5	45	140	3,4,5,7,9,12,15	5.0
	5-A	55	270	9,12,15,20,25,30,35	25.0
	5-B	45	300	3,4,5,7,9,12,15	15.0
	5	55	250	9,12,15,20,25,30,35	20.0

Table 6 (Cont.)

Trial No.	Culture	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 9, 5 days old, heated at 110.0°C. pH of milk 6.3					
1	9	37	250,000	1,2,3,4,5,7,9	3.0
	9-A	45	260,000	1,2,3,4,5,7,9	5.0
	9-B	37	240,000	1,2,3,4,5,7,9	3.0
	9	45	120,000	1,2,3,4,5,7,9	5.0
2	9	37	210,000	1,2,3,4,5,7,9	5.0
	9-A	45	205,000	1,2,3,4,5,7,9	5.0
	9-B	37	230,000	1,2,3,4,5,7,9	5.0
	9	45	170,000	1,2,3,4,5,7,9	7.0

optimum caused an increase in thermal resistance. The two exceptions to the trend were trial 2 with culture 1, in which the sudden change of growth temperature from below the optimum to the optimum actually caused a slight decrease in thermal resistance of the spores of the culture, and trial 2 with culture 9 in which there was no change in heat resistance. In contrast, a sudden change of growth temperature from the optimum to a lower temperature resulted in a decrease in the thermal resistance in every one of 21 comparisons. An explanation for the decrease in the heat resistance in trial 2 with culture 1 when the growth temperature was increased to the optimum may be in the difference in the spore content of the skimmilk. With spores grown at 21°C. the spore content per cc. of skimmilk was 192,000, while with the spores grown at 37°C. the spore content was only 15,000 per cc. The explanation, however, is not entirely satisfactory since a similar condition existed in trial 3 with culture 1 and in this case the change in growth temperature from below the optimum to the optimum caused an increase in thermal resistance. Similar differences in the spore content of the skimmilk occurred with other cultures but as shown previously in Tables 2 and 4, these apparently did not significantly influence the thermal resistance of the spores.

A summary of the data in Table 6 is given in Table 7. The average thermal resistance (expressed in minutes of

Table 7

Influence of a sudden change in growth temperature on the thermal resistance of spores of various bacteria (Summary of data in Table 6)

Culture	No. of trials	Growth temperature °C.	Age of culture (days)	Exposure temperature °C.	Spores per cc. of milk heated (average)	Survival (in min.) (average)	Range of survival (minutes)
1	4	21	5	114.5	163,600	4.5	3 - 7
1-A	4	37	5	114.5	76,370	5.7	3 - 10
1-B	4	21	5	114.5	320,000	7.2	5 - 10
1	4	37	5	114.5	162,500	18.5	15 - 25
2	4	21	5	116.0	41,620	6.5	5 - 7
2-A	4	37	5	116.0	162,250	16.2	10 - 20
2-B	4	21	5	116.0	30,500	5.7	3 - 10
2	4	37	5	116.0	24,000	16.2	15 - 20
3	3	21	5	110.0	1,900,000	0.0	0 - 0
3-A	3	37	5	110.0	2,933,000	4.3	3 - 5
3-B	3	21	5	110.0	1,333,000	2.0	0 - 3
3	3	37	5	110.0	2,133,000	6.0	5 - 10
4	5	21	5	116.0	11,200	5.8	5 - 7
4-A	5	37	5	116.0	9,000	14.0	10 - 15
4-B	5	21	5	116.0	12,000	9.8	7 - 15
4	5	37	5	116.0	14,800	19.0	15 - 25
5	3	45	3	120.0	150	5.0	5 - 5
5-A	3	55	3	120.0	216	21.6	20 - 25
5-B	3	45	3	120.0	333	8.3	5 - 15
5	3	55	3	120.0	183	23.3	20 - 25
9	2	37	5	110.0	230,000	4.0	3 - 5
9-A	2	45	5	110.0	232,500	5.0	5 - 5
9-B	2	37	5	110.0	235,000	4.0	3 - 5
9	2	45	5	110.0	205,000	6.0	5 - 7

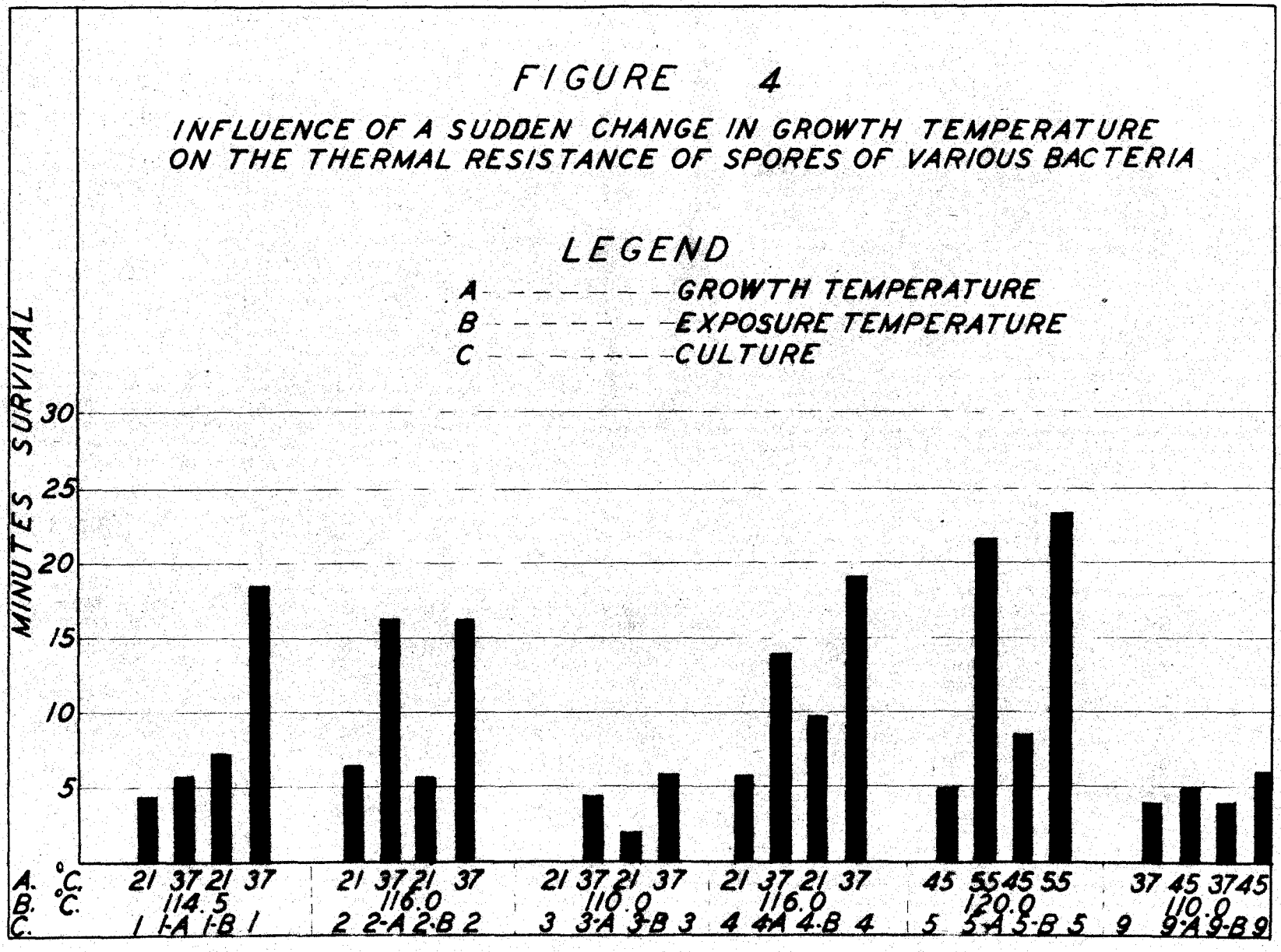
A = Change from lower to higher growth temperature.
B = Change from higher to lower growth temperature.

survival) of the different cultures shows the same influence of a sudden change in growth temperature as do the individual trials. Typical of these changes is that obtained with culture 2. When grown at 21°C., culture 2 had an average survival for the spores of 6.5 minutes at 116.0°C. but when a transfer, made at the same time as that grown at 21°C., was grown at 37°C. the average survival of the spores was 16.2 minutes at 116.0°C. The reverse occurred when culture 2, growing at 37°C., was suddenly transferred and grown at 21°C. since the survival time of the spores decreased from 16.2 minutes to 5.7 minutes. The same general tendencies were evident with each of the six cultures. The extent of the average increase or decrease in thermal resistance varied with the individual cultures, but the change in resistance was consistent and usually appreciable. These results further substantiate the results presented in Tables 2 and 3 that growth temperatures below the optimum decrease thermal resistance.

From the data given in Table 7 it appears that the spores of cultures grown for some time at the higher or optimum growth temperature were more resistant to heat than the spores of cultures grown for only one generation at the same temperature. This is perhaps more clearly demonstrated in Figure 4 where it is seen that, with the exception of culture 2, all cultures were more resistant to heat when grown for several generations at their optimum temperatures than

FIGURE 4

INFLUENCE OF A SUDDEN CHANGE IN GROWTH TEMPERATURE ON THE THERMAL RESISTANCE OF SPORES OF VARIOUS BACTERIA



when grown for only one generation. When evaluating this observation it should be pointed out that all cultures with the letters A or B after the culture number (e. g., 1-A, 1-B, 2-A, or 2-B) represent only one generation at the specific growth temperature. Culture 1 when grown for several generations at 37°C. produced spores with an average thermal resistance of 18.5 minutes but when grown for only one generation at the same temperature (culture 1-A) the average thermal resistance of the spores was only 5.7 minutes. This same tendency was shown by cultures 3, 4, 5, and 9, but to a lesser degree. With culture 2, however, spores produced after only one generation at 37°C. exhibited the same resistance to heat as did the spores from cultures grown several generations at 37°C. In contrast there appeared to be some tendency for the cultures grown for only one generation at a temperature below the optimum to produce spores with a greater thermal resistance than those grown for several generations at the same temperatures. This tendency appeared with cultures 1, 3, 4, and 5. Culture 2, however, when grown for one generation at a temperature below the optimum produced spores with a lower thermal resistance than the spores from cultures grown for several generations at the same temperature. Spores of culture 9 showed no difference in a comparison of this kind.

It is well to note in Table 7 that the average spore contents of the spore suspensions, in comparative tri-

als, were approximately the same and that the only variable factor influencing growth or resistance to heat was the growth temperature.

Effect of continued growth of various bacteria at a changed growth temperature on the thermal resistance of the spores

Observations made when there was a sudden change in growth temperature suggested that spores of cultures grown for some time at the optimum temperature were more resistant to heat than spores of cultures grown for only one generation at that temperature. In order to measure this tendency, cultures which had been suddenly changed to a lower or to a higher growth temperature were carried through a series of transfers at that temperature and then tested for heat resistance.

Table 8 shows the thermal resistance of the spores after the cultures had been carried through a series of from 5 to 11 transfers or generations at the changed growth temperature. In addition there is given, for each culture, the average thermal resistance of the spores when the culture was originally tested (data taken from Table 3) and also the average thermal resistance of the spores when the culture had grown only one generation after changing the growth temperature (data taken from Table 7). It should be noted that when the cultures were originally tested they had been carried

Table 8

Effect of continued growth of various bacteria at a changed growth temperature on the thermal resistance of the spores

No. of tr.-ans-fers	Growth temper-ature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)	Survival original culture (1) Several transfers (ave.)	Survival original culture (2) One transfer (ave.)
Culture 1-A, 5 days old, heated at 114.5°C. pH of milk 6.3						
7	37	65,000	3,5,7,10,15,20,25	15.0	16.9	5.7
Culture 1-B, 5 days old, heated at 114.5°C. pH of milk 6.3						
7	21	71,000	2,3,4,5,7,9,12	3.0	4.6	7.2
Culture 2-A, 5 days old, heated at 116.0°C. pH of milk 6.3						
5	37	100,000	7,9,12,15,20,25,30	20.0	19.9	16.2
Culture 2-B, 5 days old, heated at 116.0°C. pH of milk 6.3						
5	21	120,000	3,4,5,7,9,12,15	7.0	8.5	5.7
Culture 3-A, 5 days old, heated at 110.0°C. pH of milk 6.4						
7	37	2,000,000	1,2,3,4,5,7,9	5.0	3.0	4.3
Culture 3-B, 5 days old, heated at 110.0°C. pH of milk 6.4						
7	21	1,500,000	1,2,3,4,5,7,9	3.0	0.6	2.0

Table 8 (Cont.)

No. of trans-fers	Growth temper-ature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)	Survival original culture (1) Several transfers (ave.)	Survival original culture (2) One transfer (ave.)
Culture 4-A, 5 days old, heated at 116.0°C. pH of milk 6.3						
11	37	4,000	9,12,15,20,25,30,35	15.0	18.8	14.0
Culture 4-B, 5 days old, heated at 116.0°C. pH of milk 6.3						
11	21	20,000	3,4,5,7,9,12,15	3.0	6.3	9.8
Culture 5-A, 3 days old, heated at 120.0°C. pH of milk 6.4						
5	55	100	9,12,15,20,25,30,35	25.0	23.6	21.6
Culture 5-B, 3 days old, heated at 120.0°C. pH of milk 6.4						
5	45	200	3,4,5,7,9,12,15	5.0	6.1	8.3

A = Change from lower to higher growth temperature.
B = Change from higher to lower growth temperature.

(1) Data taken from Table 3.
(2) Data taken from Table 7.

through at least three generations.

Results presented in Table 8 indicate that the average thermal resistance of the spores of the various cultures, when grown for a period at a changed growth temperature, tended to approximate the average thermal resistance of the spores of the culture when originally tested after growth at the same temperature. The spores of culture 1-A, which was culture 1 grown at 21°C. and then changed to a temperature of 37°C., had a thermal resistance of 5.7 minutes after one generation, but after 7 generations the thermal resistance had increased to 15.0 minutes or almost the same thermal resistance as that exhibited by the spores of the culture when originally tested after growth at 37°C. Culture 1-B, which was culture 1 grown at 37°C. and then changed to a temperature of 21°C., produced spores which had an average thermal resistance of 7.2 minutes after one generation, but after 7 generations the thermal resistance had decreased to 3.0 minutes which was less than that of the spores of the cultures when originally tested after growth at 21°C. Cultures 1-A and 4-A, after 7 and 11 generations, respectively, at the changed growth temperature, produced spores which had a thermal resistance almost equal to that of the spores of the cultures when originally tested after growth at 37°C. Spores of cultures 3-A and 5-A, after 7 and 3 generations, respectively, exceeded slightly the thermal resistance of

the spores of the cultures originally tested after growth at 37°C., and spores of culture 2-A, after 5 generations, equaled the thermal resistance of the spores of the culture when originally tested after growth at 37°C. In contrast, spores of cultures 1-B, 2-B, 4-B, and 5-B, after 7, 5, 11, and 5 generations, respectively, were lower in thermal resistance than the spores of the cultures when originally tested after growth at 21°C. Spores of culture 3-B, however, after 7 generations, were not quite as low in thermal resistance as the spores of the culture when originally tested after growth at 21°C.; in fact, their thermal resistance was slightly increased.

The results given in Table 8 indicate that continued growth at a changed growth temperature generally resulted in a thermal resistance approximating that of the culture when originally tested after growth at the same temperature. Apparently the cultures, after a period of time, had become acclimatized and the growth temperature exerted its influence on the heat resisting ability of the spores produced.

Comparative thermal resistance of moist and freshly dried spores

The results already presented were obtained with moist spores. Since spores are frequently present in a dried state under practical conditions, a comparison was made of the thermal resistance of moist and freshly dried spores. Only spores from the same growth were compared. When tested for heat resistance the dried spores were three days older than the moist spores because of the drying procedure and the method of determining the spore content that were used.

The method employed in preparing the spores for the comparative trials was as follows: a series of streaks of the culture to be studied was made on the surface of nutrient or beef infusion agar in a petri dish and the dish incubated at the same temperature as the culture from which it was transferred, for either 3 or 5 days depending on the incubation temperature (3 days at 55°C., and 5 days at 21°, 37°, or 45°C.). A portion of the growth containing the moist spores was transferred directly to 60 cc. of sterile skimmilk and the spore preparation made and tested for heat resistance and spore content as outlined under "Methods". The remaining portion of the growth on the agar was scraped into a sterile petri dish and dried over calcium chloride in a partial vacuum at 37°C. for 24 hours. The dried material was ground in a sterile mortar and well mixed with sterile powdered lactose.

The spore content of the powder was determined as outlined under "Methods". Knowing the spore content of moist spores per cubic centimeter of skimmilk used for the trials and the content of dried spores per 0.01 gram of spore powder, the amount of spore powder used per 60 cc. of skimmilk was adjusted so that the comparative trials with moist and dried spores were carried out with approximately the same number of spores per cubic centimeter.

In several instances it was necessary to make two or three comparisons before satisfactory spore counts were secured. In these comparisons it was noted that even considerable variations in the spore contents of the skimmilk used in the heat resistance testsmade no discernible difference in the length of time the spores survived the heat treatment.

When spores were obtained from cultures at two different growth temperatures, the higher of the two temperatures was always the optimum temperature. When three growth temperatures were used, the middle temperature was always the optimum.

The results of 22 comparisons of moist and dried spores, using eight different cultures, are presented in Table 9. Nine of the 22 trials showed a greater resistance for the moist spores, 4 for the dried spores, and 9 gave no advantage to either type of spore.

Moist spores of culture 1 grown at 21°C., culture 2

Table 9

Comparative thermal resistance of moist and freshly dried spores

Type of spore	Growth temperature °C.	Age of spores (days)	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)	Survival original culture (ave.)
Culture 1, heated at 116.0°C. pH of milk 6.3						
Moist	21	5	160,000	3,5,7,10,15,20,25	7.0	4.6
Dried	21	8	160,000	3,5,7,10,15,20,25	5.0	
Moist	37	5	4,000	3,5,7,10,15,20,25	10.0	16.9
Dried	37	8	4,000	3,5,7,10,15,20,25	10.0	
Culture 2, heated at 116.0°C. pH of milk 6.3						
Moist	21	5	2,000	3,5,7,10,15,20,25	3.0	8.5
Dried	21	8	2,000	3,5,7,10,15,20,25	3.0	
Moist	37	5	3,000	7,9,12,15,20,25,30	15.0	19.9
Dried	37	8	3,000	7,9,12,15,20,25,30	7.0	
Moist	45	5	2,500	3,5,7,10,15,20,25	3.0	3.0
Dried	45	8	2,500	3,5,7,10,15,20,25	0.0	
Culture 4, heated at 116.0°C. pH of milk 6.3						
Moist	21	5	125,000	3,5,7,10,15,20,25	10.0	6.3
Dried	21	8	125,000	3,5,7,10,15,20,25	5.0	
Moist	37	5	100,000	5,7,9,12,15,20,25	20.0	18.8
Dried	37	8	100,000	5,7,9,12,15,20,25	15.0	
Moist	45	5	40,000	3,5,7,10,15,20,25	10.0	6.0
Dried	45	8	40,000	3,5,7,10,15,20,25	15.0	
Culture 5, heated at 120.0°C. pH of milk 6.3						
Moist	45	3	400	3,4,5,7,9,12,15	7.0	6.1
Dried	45	6	400	3,4,5,7,9,12,15	7.0	
Moist	55	3	500	3,5,7,10,15,20,25	15.0	23.6
Dried	55	6	500	3,5,7,10,15,20,25	10.0	

Table 9 (Cont.)

Type of spore	Growth temperature °C.	Age of spores (days)	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)	Survival original culture (ave.)*
Culture 7, heated at 106.0°C. pH of milk 6.3						
Moist	21	5	2,000,000	1,2,3,4,5,7,9	3.0	3.0
Dried	21	8	2,000,000	1,2,3,4,5,7,9	3.0	
Moist	37	5	1,000,000	2,3,4,5,7,9,12	7.0	7.0
Dried	37	8	1,000,000	2,3,4,5,7,9,12	7.0	
Moist	45	5	1,500,000	2,3,4,5,7,9,12	7.0	7.0
Dried	45	8	1,500,000	2,3,4,5,7,9,12	7.0	
Culture 9, heated at 110.0°C. pH of milk 6.3						
Moist	37	5	300,000	1,2,3,4,5,7,9	5.0	4.3
Dried	37	8	300,000	1,2,3,4,5,7,9	3.0	
Moist	45	5	280,000	2,3,4,5,7,9,12	7.0	5.3
Dried	45	8	280,000	2,3,4,5,7,9,12	3.0	
Moist	55	3	76,000	2,3,4,5,7,9,12	5.0	4.0
Dried	55	6	76,000	2,3,4,5,7,9,12	3.0	
Culture 10, heated at 116.0°C. pH of milk 6.3						
Moist	45	3	600	3,5,7,10,15,20,25	10.0	10.0
Dried	45	6	600	3,5,7,10,15,20,25	15.0	
Moist	55	3	800	15,20,25,30,35,40,45	35.0	30.0
Dried	55	6	800	15,20,25,30,35,40,45	35.0	
Culture 11, heated at 116.0°C. pH of milk 6.3						
Moist	45	3	900	15,20,25,30,35,40,45	25.0	25.0
Dried	45	6	900	15,20,25,30,35,40,45	25.0	
Moist	55	3	700	15,20,25,30,35,40,45	30.0	32.5
Dried	55	6	700	15,20,25,30,35,40,45	35.0	

Table 9 (Cont.)

Type of spore	Growth temperature °C.	Age of spores (days)	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in minutes)	Survival original culture (ave.)*
Culture 12, heated at 116.0°C. pH of milk 6.3						
Moist	45	3	9,500	5,7,9,12,15,20,25	5.0	10.0
Dried	45	6	9,500	5,7,9,12,15,20,25	5.0	
Moist	55	3	8,300	15,20,25,30,35,40,45	30.0	30.0
Dried	55	6	8,300	15,20,25,30,35,40,45	35.0	

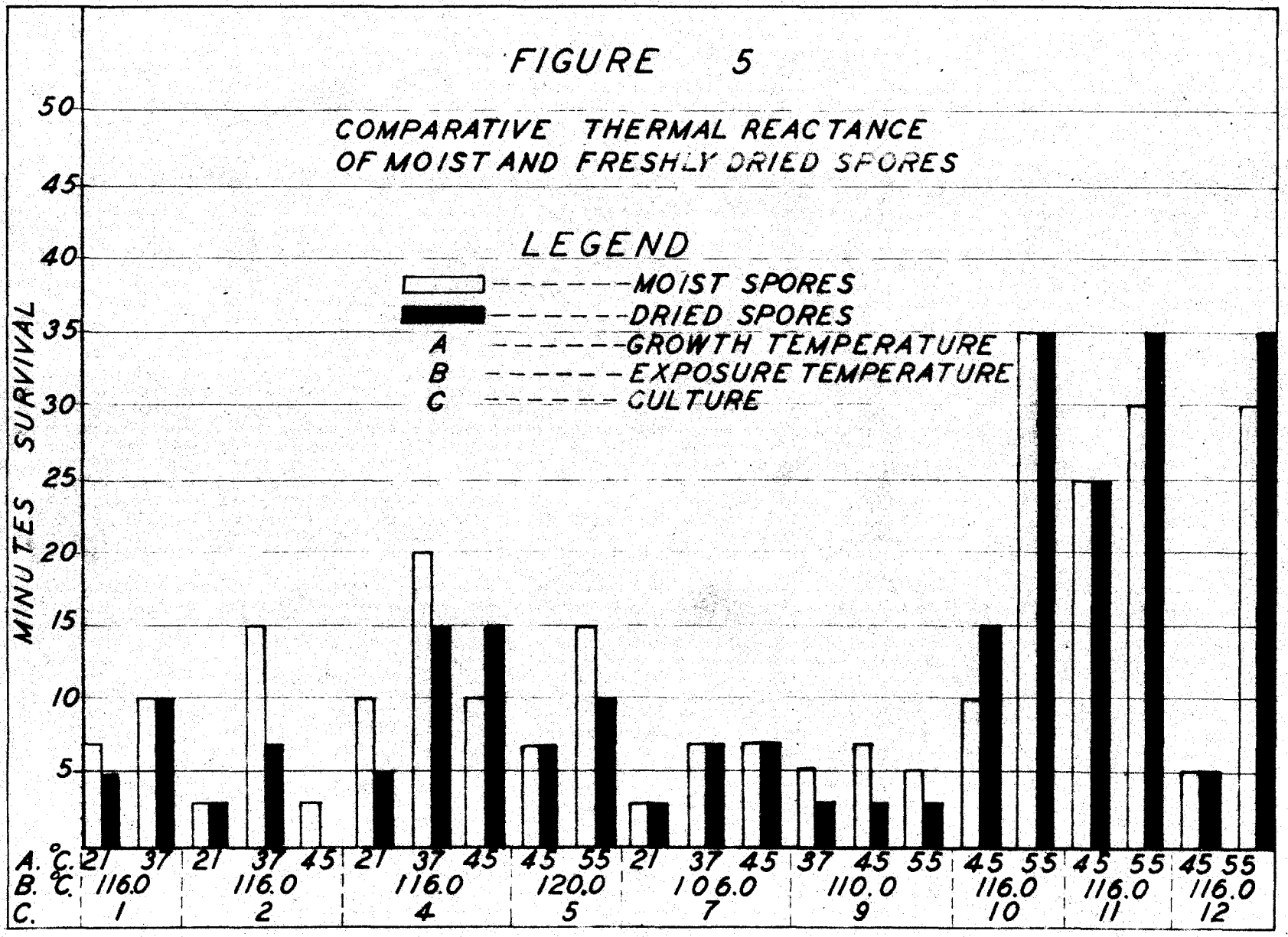
*Data taken from Tables 3 and 5.

grown at 37° or 45°C., culture 4 grown at 21° or 37°C., culture 5 grown at 55°C., and culture 9 grown at 37°, 45°, or 55°C. showed a greater resistance to heat of 2.0, 8.0, 3.0, 5.0, 5.0, 5.0, 2.0, 4.0, and 2.0 minutes, respectively, than did the freshly dried spores of the same cultures. In contrast, the freshly dried spores of culture 4 grown at 45°C., culture 10 grown at 45°C., culture 11 grown at 55°C., and culture 12 grown at 55°C., showed a greater resistance to heat of 5.0, 5.0, 5.0, and 5.0 minutes, respectively, than did the moist spores of the same cultures. There was no difference in the thermal resistance of the two types of spore when culture 1 was grown at 37°C., culture 2 grown at 21°C., culture 5 grown at 45°C., culture 7 grown at 21°, 37°, or 45°C., culture 10 grown at 55°C., and culture 11 and 12 grown at 45°C. Although the results varied and showed no great uniformity as to which type of spore had the greater thermal resistance, the tendency appeared to be for the moist spores to be the more resistant.

The data are expressed graphically in Figure 5. This figure shows that growth below the optimum temperature resulted in both moist and dried spores with a lowered thermal resistance. In every one of the nine comparisons the moist spores showed a lower thermal resistance when grown at temperatures below the optimum than when grown at the optimum. In eight of nine comparisons dried spores also showed a lower resistance when grown below the optimum than when grown at the

FIGURE 5

COMPARATIVE THERMAL REACTANCE OF MOIST AND FRESHLY DRIED SPORES



optimum. The dried spores of culture 9 exhibited the same resistance to heat at all three growth temperatures. These general results not only verify the data presented in Tables 3, 5, and 7 but indicate further that the freshly dried spores are likewise decreased in thermal resistance when developed by cultures grown at a temperature below their optimum.

Cultures 2, 4, 7, and 9 were the only cultures which grew at three different temperatures and from which dried spores were prepared. An analysis of the data in Table 9 shows that moist and freshly dried spores of culture 2 and moist spores of cultures 4 and 9, produced at a growth temperature above the optimum, were lower in thermal resistance than similar spores grown at the optimum temperature. Freshly dried spores of cultures 4 and 9 and moist and freshly dried spores of culture 7, however, had the same resistance when grown at a temperature just above the optimum as when grown at the optimum. These observations substantiate the data (Tables 4 and 5) indicating that growth temperatures above the optimum tend to decrease the thermal resistance of moist spores. No explanation can be given for the freshly dried spores not exhibiting the same tendency.

Cultures 1, 2, 4, and 5 had been carried for approximately 150, 120, 90, and 60 days, respectively, on artificial culture media at the time the comparison of the heat resistance of moist and freshly dried spores was made. Table 9

shows that moist and freshly dried spores of culture 1, grown at the optimum temperature, exhibited a lower heat resistance than the moist spores of the culture when originally tested for heat resistance. With the spores of culture 1 the temperature of exposure used when originally tested was 114.5°C., while the data presented in Table 9 for the moist and freshly dried spores was obtained with an exposure temperature of 116.0°C. Just how much of the higher heat resistance of the moist spores of the cultures when originally tested should be attributed to the difference in exposure temperatures can not be accurately determined. It does not seem, however, that all of the difference in resistance (6.9 minutes) could be due to only 1.5°C. difference in the temperature of exposure. Both moist and freshly dried spores of cultures 2 and 5, grown at their optimum temperatures, also showed an appreciably lower heat resistance than the moist spores of the cultures when originally tested for thermal resistance at the same temperature. Moist spores of culture 4, grown at the optimum temperature, were very slightly more resistant to heat than the moist spores of the culture when originally tested at the same temperature, but the dried spores grown at the same temperature were less resistant. Growth temperatures other than the optimum gave varied results in so far as indicating any influence of either age of culture or growth on artificial culture media on the thermal resistance

of moist spores. Cultures 7, 9, 10, 11, and 12 had been carried only 20, 30, 15, 15, and 15 days, respectively, on the artificial culture media when the series of trials was run. Regardless of the growth temperatures used, 10 of 11 comparisons of moist and freshly dried spores of cultures 7, 9, 10, 11, and 12 showed no significant differences in heat resistance. Comparisons of the thermal resistance of the freshly dried spores with the thermal resistance of the moist spores of the cultures when originally tested gave varied results. In general, the data suggest that at the optimum growth temperature long periods of growth of cultures on artificial culture media tended to decrease the thermal resistance of both moist and freshly dried spores.

Influence of age on the thermal resistance of dried spores

Spores were dried by the procedure outlined under "Methods" and thermal resistance trials run when the spore powders were from 5 to 8 days old (freshly dried spores) and again when they were from 47 to 69 days old. The spore powders were held in a desiccator at 21°C. over calcium chloride.

Eighteen comparative trials were conducted, using eight different cultures, and Table 10 shows the results obtained. Freshly dried spores of culture 2 grown at 37°C., culture 10 grown at 55°C., and culture 12 grown at 55°C.,

Table 10

Influence of age on the thermal resistance of dried spores

Age of spores (days)	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)	Survival* original culture (in min.) (average)
	Culture 2, heated at 116.0°C. pH of milk 6.3				
7	21	4,000	3,5,7,10,15,20,25	3.0	8.5
69	21	4,000	3,5,7,10,15,20,25	10.0	
7	37	4,000	7,9,12,15,20,25,30	25.0	19.9
69	37	4,000	7,9,12,15,20,25,30	20.0	
	Culture 4, heated at 116.0°C. pH of milk 6.3				
8	21	10,000	3,5,7,10,15,20,25	10.0	6.3
68	21	10,000	3,5,7,10,15,20,25	15.0	
8	37	10,000	5,7,9,12,15,20,25	7.0	18.8
68	37	10,000	5,7,9,12,15,20,25	15.0	
8	45	10,000	3,5,7,10,15,20,25	5.0	6.0
68	45	10,000	3,5,7,10,15,20,25	7.0	
	Culture 5, heated at 120.0°C. pH of milk 6.3				
5	55	100	3,5,7,10,15,20,25	10.0	23.6
66	55	100	3,5,7,10,15,20,25	10.0	
	Culture 7, heated at 106.0°C. pH of milk 6.3				
7	21	20,000	1,2,3,4,5,7,9	3.0	3.0
68	21	20,000	1,2,3,4,5,7,9	3.0	
7	37	20,000	2,3,4,5,7,9,12	5.0	7.0
68	37	20,000	2,3,4,5,7,9,12	5.0	
7	45	20,000	2,3,4,5,7,9,12	5.0	7.0
68	45	20,000	2,3,4,5,7,9,12	7.0	

Table 10 (Cont.)

Age of spores (days)	Growth temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)	Survival* original culture (in min.) (average)
	Culture 8, heated at 106.0°C. pH of milk 6.3				
8	21	20,000	1,2,3,4,5,7,9	0.0	0.0
69	21	20,000	1,2,3,4,5,7,9	3.0	
8	37	20,000	1,2,3,4,5,7,9	3.0	3.0
69	37	20,000	1,2,3,4,5,7,9	3.0	
8	45	20,000	1,2,3,4,5,7,9	0.0	0.0
69	45	20,000	1,2,3,4,5,7,9	3.0	
	Culture 9, heated at 110.0°C. pH of milk 6.3				
7	37	1,000	2,3,4,5,7,9,12	5.0	4.3
68	37	1,000	2,3,4,5,7,9,12	5.0	
7	45	1,000	2,3,4,5,7,9,12	5.0	5.3
68	45	1,000	2,3,4,5,7,9,12	7.0	
7	55	1,000	2,3,4,5,7,9,12	5.0	4.0
68	55	1,000	2,3,4,5,7,9,12	5.0	
	Culture 10, heated at 116.0°C. pH of milk 6.3				
5	45	100	3,5,7,10,15,20,25	0.0	10.0
47	45	100	3,5,7,10,15,20,25	5.0	
5	55	100	15,20,25,30,35,40,45	25.0	30.0
47	55	100	15,20,25,30,35,40,45	20.0	
	Culture 12, heated at 116.0°C. pH of milk 6.3				
5	55	40	15,20,25,30,35,40,45	25.0	30.0
47	55	40	15,20,25,30,35,40,45	15.0	

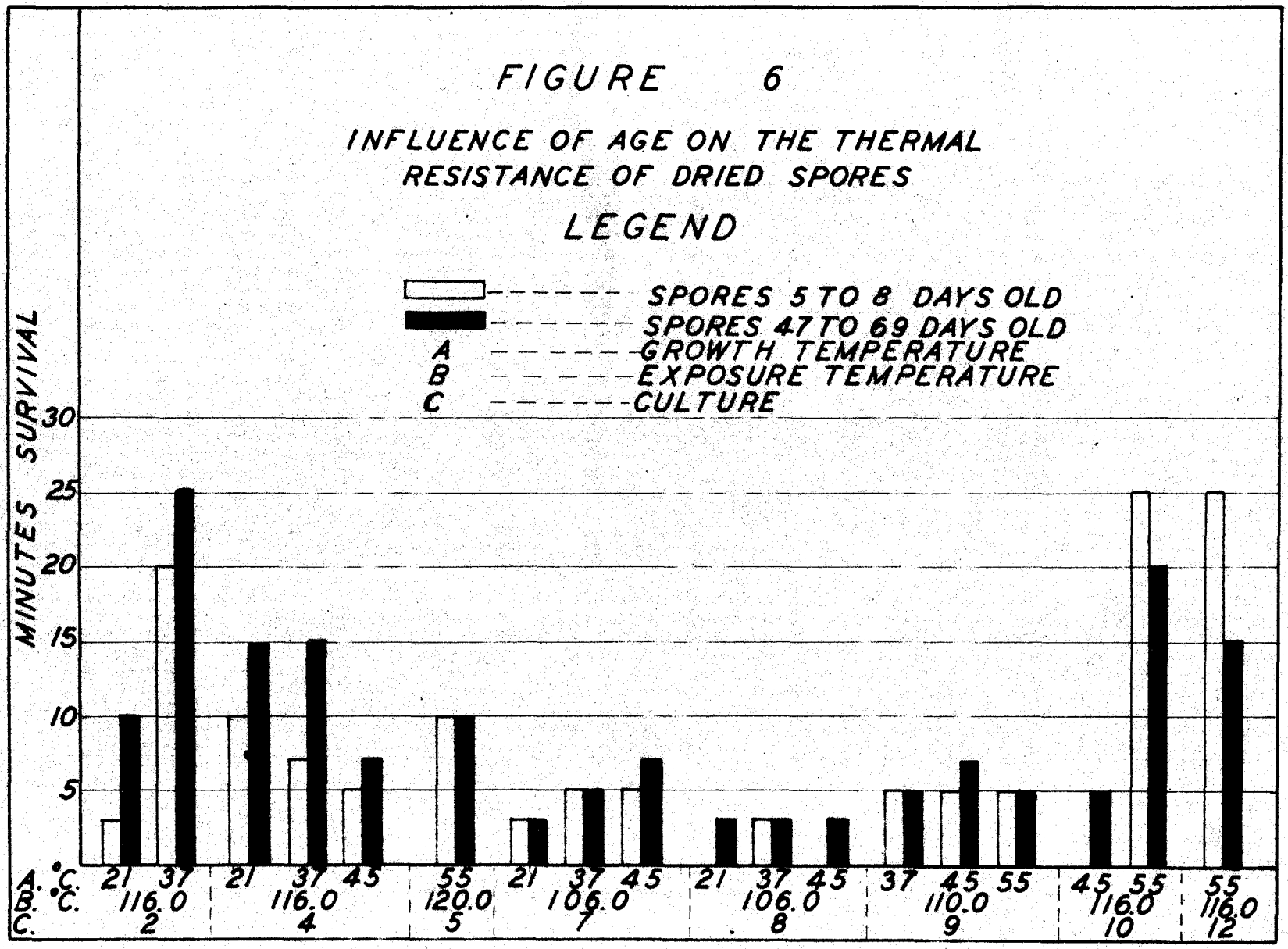
*Data taken from Tables 3 and 5.

resisted the temperature of exposure used for 5 minutes longer than the aged spores. Aged spores of culture 2 grown at 21°C., culture 4 grown at 21°, 37°, or 45°C., culture 7 grown at 45°C., culture 8 grown at 21° or 45°C., culture 9 grown at 45°C., and culture 10 grown at 45°C. possessed a greater thermal resistance of 7.0, 5.0, 8.0, 2.0, 2.0, 5.0, 5.0, 2.0, and 5.0 minutes, respectively, than the freshly dried spores. There was no difference in the thermal resistance of the freshly dried and aged spores when culture 5 was grown at 55°C., culture 7 at 21° or 37°C., culture 8 at 37°C., and culture 9 at 37° or 55°C.

Figure 6 shows the differences in thermal resistance of the spores of different ages from cultures grown at different temperatures. The three trials in which freshly dried spores had the greater thermal resistance represented three different cultures (cultures 2, 10, and 12), each grown at its optimum growth temperature, but 2 of the 9 trials showing a greater thermal resistance for the aged spores were also different cultures, (cultures 4 and 9), each grown at its optimum temperature. Only aged spores from culture 4 showed a greater resistance to heat than the freshly dried spores at all growth temperatures. With cultures 7, 8, and 9, any difference in resistance was in favor of the aged spores, but there was no correlation with any specific growth temperature. Apparently, with the organisms studied, any influence of age on the thermal resistance of dried spores made the aged spores

FIGURE 6

INFLUENCE OF AGE ON THE THERMAL RESISTANCE OF DRIED SPORES



more heat resistant but the influence was not correlated with temperature of growth.

Comparisons between the thermal resistance of the dried spores (freshly dried or aged) and the resistance of the moist spores of the same cultures when originally tested for heat resistance, at the same temperature, were possible in 36 instances from the data in Table 10. Twelve comparisons showed a greater heat resistance for the dried spores, 4 for the freshly dried spores, and 8 for the aged spores. Sixteen comparisons showed a lower heat resistance for the dried spores, 10 with the freshly dried spores, and 6 with the aged spores. In 8 comparisons the resistance of the dried spores and the moist spores were the same, 4 with freshly dried spores and 4 with aged spores. These results indicate that, in general, the thermal resistance of dried spores, whether fresh or aged, does not differ greatly from the resistance of moist spores. If any greater thermal resistance can be attributed to either type of spore, the data suggest that moist spores have the greater thermal resistance which is in agreement with the results presented in Table 9. A notable example of the greater resistance of moist spores is given by culture 5 as shown in Table 10. Both the freshly dried and aged spores of culture 5 had a thermal resistance of 10 minutes at 120.0°C., while the fresh moist spores averaged 23.5 minutes survival at the same temperature. The difference in resistance may

be attributed either to desiccation of the spores or to the culture having been carried for approximately 60 days on artificial culture media before the spores were grown for drying. It is possible both factors were involved.

Effect of continued growth of various bacteria on artificial culture media on the thermal resistance of the spores

Some of the results presented in Tables 9 and 10 suggest that prolonged growth on artificial culture media may reduce the thermal resistance of the spores of certain bacteria. The effect of continued growth on artificial culture media was determined by comparing the average thermal resistance of the spores of the cultures when originally tested with the resistance of the spores of the same cultures after a number of transfers on artificial media. The cultures had been carried through a series of transfers (5 to 56) on artificial culture media during from 15 to 197 days. The data on the thermal resistance of the spores of the cultures when originally tested were taken from Tables 3 and 5.

Data for the comparisons are given in Table 11. Culture 1, after being carried through a series of transfers (39 transfers) on artificial culture media, for 197 days, produced spores at both 21° and 37°C. which exhibited a lower resistance to heat than spores of the culture when originally tested after growth at the same temperatures. An exposure

Table 11

Effect of continued growth of various bacteria on artificial media on the thermal resistance of the spores

Growth temperature °C.	Growth on artificial media (days)	Number of transfers	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)	Survival original culture (ave.)
	Culture 1, heated at 116.0°C. pH of milk 6.4					
21	197	39	94,000	3,5,7,10,15,20,25	3.0	4.6
37	197	39	70,000	3,5,7,10,15,20,25	15.0	16.9
	Culture 2, heated at 116.0°C. pH of milk 6.4					
21	168	33	13,000	3,5,7,10,15,20,25	3.0	8.5
37	168	33	11,000	3,5,7,10,15,20,25	10.0	19.9
45	168	56	1,300	2,3,4,5,7,9,12	3.0	5.0
	Culture 4, heated at 116.0°C. pH of milk 6.4					
21	130	26	163,000	3,5,7,10,15,20,25	3.0	6.3
37	130	26	171,000	3,5,7,10,15,20,25	15.0	18.8
45	130	43	11,000	3,5,7,10,15,20,25	3.0	6.0
	Culture 5, heated at 120.0°C. pH of milk 6.4					
45	92	30	310	3,5,7,10,15,20,25	5.0	6.1
55	92	30	280	9,12,15,20,25,30,35	15.0	23.6
	Culture 9, heated at 110.0°C. pH of milk 6.4					
37	34	7	21,000	3,5,7,10,15,20,25	3.0	4.3
45	34	11	10,000	3,5,7,10,15,20,25	5.0	5.0
55	34	11	12,000	3,5,7,10,15,20,25	3.0	4.0
	Culture 10, heated at 116.0°C. pH of milk 6.4					
45	15	5	410	3,5,7,10,15,20,25	10.0	10.0
55	15	5	500	15,20,25,30,35,40,45	30.0	30.0

Table 11 (Cont.)

Growth temperature °C.	Growth on artificial media (days)	Number of transfers	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)	Survival original culture (ave.)*
Culture 11, heated at 116.0°C. pH of milk 6.4						
45	15	5	16,000	15,20,25,30,35,40,45	25.0	25.0
55	15	5	12,800	15,20,25,30,35,40,45	35.0	32.5
Culture 12, heated at 116.0°C. pH of milk 6.4						
45	15	5	12,300	15,20,25,30,35,40,45	15.0	10.0
55	15	5	10,400	15,20,25,30,35,40,45	30.0	30.0

* Data taken from Tables 3 and 5.

temperature of 114.5°C. was employed when the culture was originally tested while after continued growth on artificial culture media a temperature of 116.0°C. was used. The difference of 1.5°C. in exposure temperature might partially explain the lower thermal resistance of the spores from the cultures carried for a considerable period on artificial culture media. Cultures 2, 4, 5, and 9 when carried through a series of transfers on artificial culture media for 168, 130, 92, and 34 days, respectively, produced spores at all growth temperatures which also showed a lower thermal resistance than the spores of the same cultures when originally tested, at the same exposure temperature, after growth at identical temperatures. The differences in heat resistances ranged from 1.0 to 10.0 minutes with the various cultures. Culture 11 when carried through 5 transfers (15 days) on artificial media at 55°C. produced spores which exhibited a slightly greater resistance to heat (2.5 minutes) than did the spores of the culture when originally tested at the same temperature. The same was true of culture 12 after 5 transfers (15 days) on artificial media at 45°C. With culture 10 at 45° or 55°C., culture 11 at 45°C., and culture 12 at 55°C., the heat resistance of the spores developed after 5 transfers (15 days) on artificial media was the same as that of the spores of the cultures when originally tested, at the same temperature, after growth at identical temperatures. Apparently, with

some organisms, comparatively short periods of growth on artificial culture media have no significant influence on the thermal resistance of the spores produced. There was no evident relationship between the number of transfers made and a change in thermal resistance with any of the cultures studied. The data suggest that, with some organisms, a long period of growth on artificial culture media rather than the number of transfers made was the factor causing a decrease in the heat resistance of the spores. It can not be said, however, that any decrease in thermal resistance was proportional to the extent of the period of growth on artificial culture media since the data obtained do not show the thermal resistance of the spores of the respective cultures when grown for various periods on the media at the different temperatures. The extent of the decreases at the end of the growth periods used varied with the different cultures at the different growth temperatures, but in all cases was definite.

The results indicate that, with the cultures studied, prolonged periods of growth on artificial culture media tended to decrease their resistance to heat.

Relation of thermal resistance to growth and exposure temperatures

It is apparent that in any one suspension medium the thermal resistance of a given number of spores of a particular species of bacteria depends on two factors, temperature and time of exposure to heat. Since trials on thermal

resistance of the various cultures used in the study were planned to secure data on the influence of the growth temperatures it was thought advisable to make an effort to correlate, if possible, the thermal resistance of the spores with both growth and exposure temperatures. With this in mind, an entirely new series of thermal resistance trials was run with several of the cultures which had exhibited considerable resistance to heat. Spores of the cultures grown at different temperatures were exposed to temperatures of 104.0°, 108.0°, 112.0°, 116.0°, and 120.0°C. The results of these trials are presented in Table 12. The relation of thermal resistance to growth temperature and exposure temperature of cultures 1, 2, 4, 5, 9, 10, 11, and 12, are better shown graphically in Figures 7, 8, 9, 10, 11, 12, 13, and 14, respectively. The values for the survival in minutes have been plotted against exposure temperatures. The data were plotted in this manner to determine the variations in the periods of survival with both the growth temperatures and the temperatures of exposure. The graphs clearly show that the spores of each of the cultures became progressively less resistant to heat when exposed to higher temperatures. In other words, the survival time varied inversely as some function of the temperature of exposure. Differences in thermal resistance of the same cultures grown at different temperatures were smallest at the highest exposure temperature.

Table 12

Relation of thermal resistance
to growth and exposure temperatures

Growth temperature °C.	Age of culture (days)	Exposure temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 1. pH of milk 6.4					
21	5	104.0	80,000	130,135,140,145,150,155,160	135.0
37	5	104.0	14,000	130,135,140,145,150,155,160	160.0
21	5	108.0	65,000	55,60,65,70,75,80,85	60.0
37	5	108.0	40,000	55,60,65,70,75,80,85	80.0
21	5	112.0	300,000	20,25,30,35,40,45,50	30.0
37	5	112.0	60,000	20,25,30,35,40,45,50	40.0
21	5	116.0	94,000	3,5,7,10,15,20,25	5.0
37	5	116.0	70,000	3,5,7,10,15,20,25	15.0
21	5	120.0	80,000	1,2,3,4,5,7,9	2.0
37	5	120.0	52,000	1,2,3,4,5,7,9	3.0
Culture 2. pH of milk 6.4					
21	5	104.0	11,000	140,150,160,170,180,190,200	150.0
37	5	104.0	10,000	140,150,160,170,180,190,200	190.0
45	3	104.0	1,000	140,150,160,170,180,190,200	150.0
21	5	108.0	17,000	70,80,90,100,110,120,130	90.0
37	5	108.0	15,000	70,80,90,100,110,120,130	120.0
45	3	108.0	4,000	60,80,90,100,110,120,130	90.0
21	5	112.0	6,000	15,20,25,30,35,40,45	20.0
37	5	112.0	5,500	25,30,35,40,45,50,55	50.0
45	3	112.0	2,000	15,20,25,30,35,40,45	20.0
21	5	116.0	13,000	3,5,7,10,15,20,25	3.0
37	5	116.0	11,000	3,5,7,10,15,20,25	10.0
45	3	116.0	3,000	3,5,7,10,15,20,25	3.0
21	5	120.0	15,500	1,2,3,4,5,7,9	1.0
37	5	120.0	14,100	1,2,3,4,5,7,9	4.0
45	3	120.0	2,500	1,2,3,4,5,7,9	1.0
Culture 4. pH of milk 6.4					
21	5	104.0	160,000	100,110,120,130,140,150,160	130.0
37	5	104.0	171,000	140,150,160,170,180,190,200	190.0
45	3	104.0	8,200	100,110,120,130,140,150,160	120.0
21	5	108.0	141,000	50,60,70,80,90,100,110	60.0

Table 12 (Cont.)

Growth temperature °C.	Age of culture (days)	Exposure temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 4. pH of milk 6.4					
37	5	108.0	193,000	70,80,90,100,110,120,130	120.0
45	3	108.0	11,000	50,60,70,80,90,100,110	60.0
21	5	112.0	192,000	15,20,25,30,35,40,45	20.0
37	5	112.0	179,000	25,30,35,40,45,50,55	50.0
45	3	112.0	5,400	15,20,25,30,35,40,45	20.0
21	5	116.0	163,000	3,5,7,10,15,20,25	3.0
37	5	116.0	177,000	3,5,7,10,15,20,25	15.0
45	3	116.0	11,400	3,5,7,10,15,20,25	3.0
21	5	120.0	176,000	1,2,3,4,5,7,9	1.0
37	5	120.0	183,000	1,2,3,4,5,7,9	7.0
45	3	120.0	7,700	1,2,3,4,5,7,9	1.0
Culture 5. pH of milk 6.4					
45	3	104.0	300	100,110,120,130,140,150,160	130.0
55	3	104.0	250	160,170,180,190,200,210,220	190.0
45	3	108.0	280	90,100,110,120,130,140,150	100.0
55	3	108.0	340	130,140,150,160,170,180,190	130.0
45	3	112.0	410	20,25,30,35,40,45,50	30.0
55	3	112.0	290	40,50,60,70,80,90,100	60.0
45	3	116.0	370	3,5,7,10,15,20,25	10.0
55	3	116.0	320	20,25,30,35,40,45,50	25.0
45	3	120.0	310	3,5,7,10,15,20,25	5.0
55	3	120.0	280	15,20,25,30,35,40,45	15.0
Culture 9. pH of milk 6.4					
37	5	104.0	20,000	3,5,7,10,15,20,25	10.0
45	3	104.0	17,000	3,5,7,10,15,20,25	15.0
55	3	104.0	15,000	3,5,7,10,15,20,25	10.0
37	5	108.0	100,000	3,5,7,10,15,20,25	5.0
45	3	108.0	52,000	3,5,7,10,15,20,25	10.0
55	3	108.0	12,000	3,5,7,10,15,20,25	5.0
37	5	112.0	21,000	1,2,3,4,5,7,9	2.0
45	3	112.0	11,000	1,2,3,4,5,7,9	4.0
55	3	112.0	13,000	1,2,3,4,5,7,9	2.0
37	5	116.0	31,000	1,2,3,4,5,7,9	1.0

Table 12,(Cont.)

Growth temperature °C.	Age of culture (days)	Exposure temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
			Culture 9. pH of milk 6.4		
45	3	116.0	8,700	1,2,3,4,5,7,9	2.0
55	3	116.0	7,900	1,2,3,4,5,7,9	1.0
37	5	120.0	19,000	1,2,3,4,5,7,9	0.0
45	3	120.0	16,500	1,2,3,4,5,7,9	1.0
55	3	120.0	11,500	1,2,3,4,5,7,9	0.0
			Culture 10. pH of milk 6.4		
45	3	104.0	280	100,110,120,130,140,150,160	140.0
55	3	104.0	330	160,170,180,190,200,210,220	180.0
45	3	108.0	350	90,100,110,120,130,140,150	100.0
55	3	108.0	320	130,140,150,160,170,180,190	130.0
45	3	112.0	210	40,50,60,70,80,90,100	60.0
55	3	112.0	170	40,50,60,70,80,90,100	90.0
45	3	116.0	410	3,5,7,10,15,20,25	10.0
55	3	116.0	500	15,20,25,30,35,40,45	30.0
45	3	120.0	340	3,5,7,10,15,20,25	7.0
55	3	120.0	410	3,5,7,10,15,20,25	10.0
			Culture 11. pH of milk 6.4		
45	3	104.0	20,300	100,110,120,130,140,150,160	160.0
55	3	104.0	18,000	160,170,180,190,200,210,220	200.0
45	3	108.0	10,200	90,100,110,120,130,140,150	120.0
55	3	108.0	15,000	130,140,150,160,170,180,190	140.0
45	3	112.0	11,700	40,50,60,70,80,90,100	60.0
55	3	112.0	17,400	40,50,60,70,80,90,100	90.0
45	3	116.0	14,100	3,5,7,10,15,20,25	25.0
55	3	116.0	12,800	15,20,25,30,35,40,45	35.0
45	3	120.0	8,300	3,5,7,10,15,20,25	7.0
55	3	120.0	16,500	3,5,7,10,15,20,25	10.0

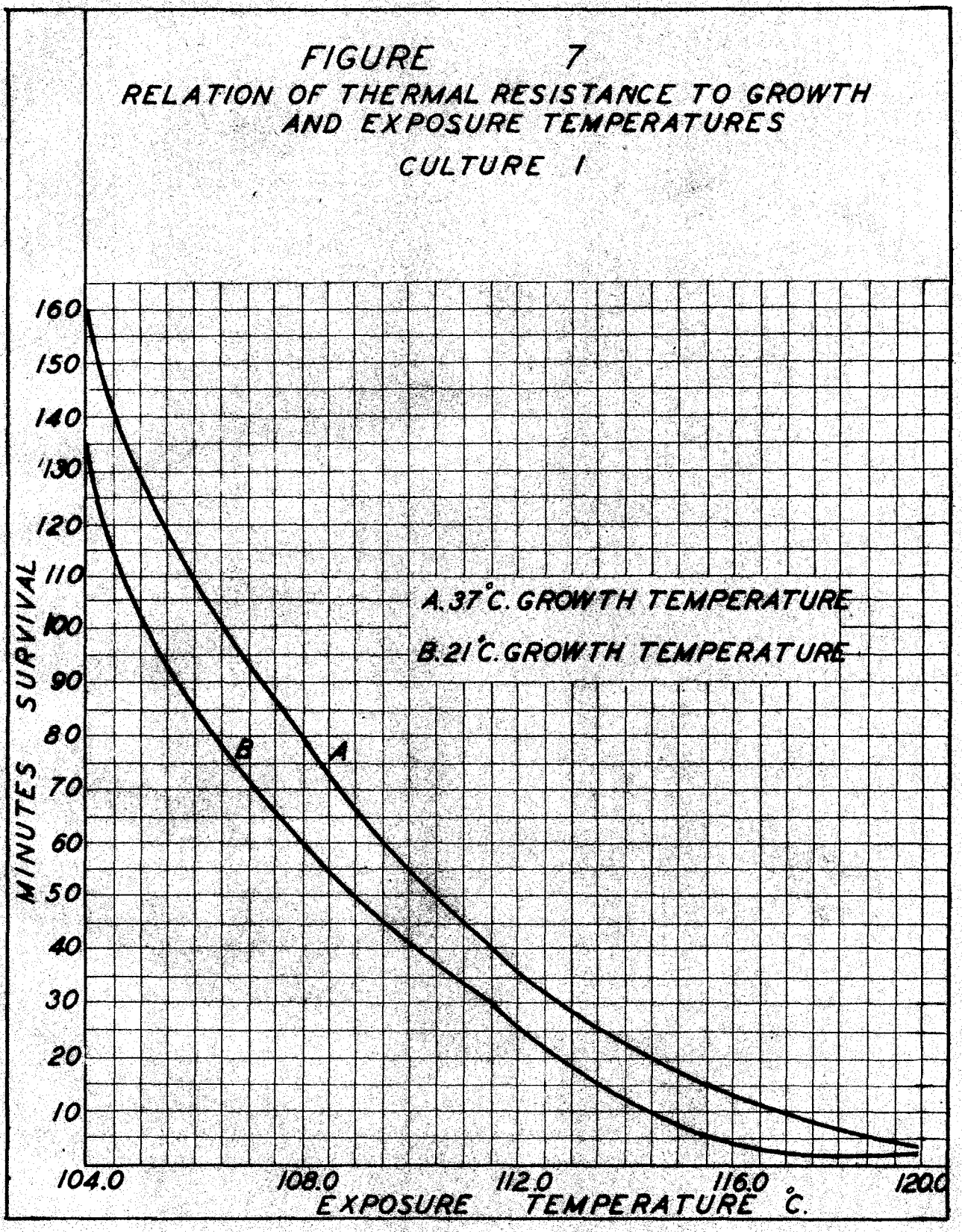
Table 12 (Cont.)

Growth temperature °C.	Age of culture (days)	Exposure temperature °C.	Spores per cc. of milk heated	Periods of heating (in minutes)	Survival (in min.)
Culture 12. pH of milk 6.4					
45	3	104.0	16,800	160,170,180,190,200,210,220	180.0
55	3	104.0	12,000	160,170,180,190,200,210,220	210.0
45	3	108.0	7,500	100,110,120,130,140,150,160	110.0
55	3	108.0	7,100	100,110,120,130,140,150,160	150.0
45	3	112.0	13,500	40,50,60,70,80,90,100	40.0
55	3	112.0	12,100	40,50,60,70,80,90,100	80.0
45	3	116.0	12,300	3,5,7,10,15,20,25	15.0
55	3	116.0	11,400	15,20,25,30,35,40,45	30.0
45	3	120.0	16,100	3,5,7,10,15,20,25	7.0
55	3	120.0	10,200	3,5,7,10,15,20,25	10.0

FIGURE 7

RELATION OF THERMAL RESISTANCE TO GROWTH AND EXPOSURE TEMPERATURES

CULTURE 1



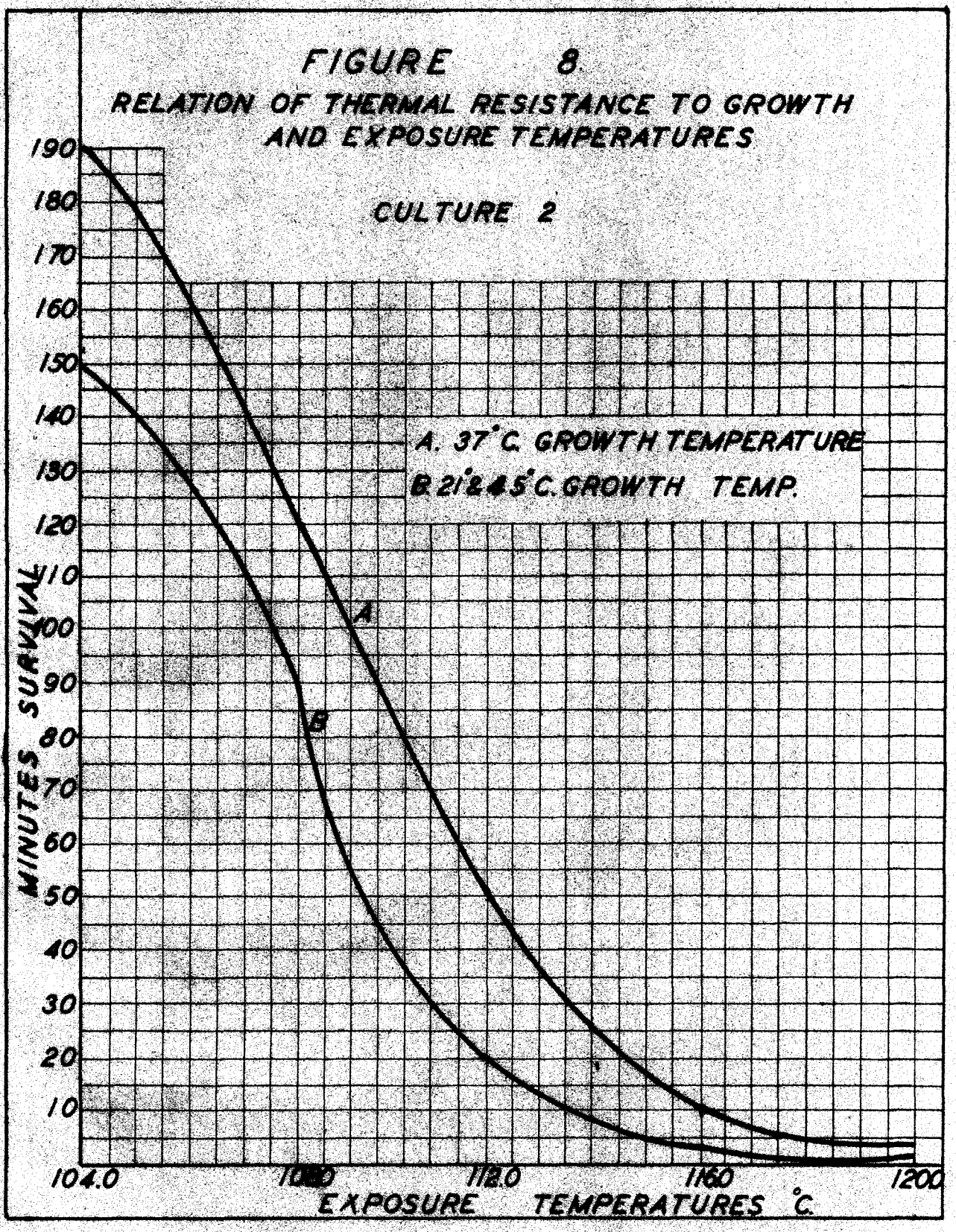


FIGURE 8.
RELATION OF THERMAL RESISTANCE TO GROWTH AND EXPOSURE TEMPERATURES
CULTURE 2
A. 37°C. GROWTH TEMPERATURE
B. 21°&45°C. GROWTH TEMP.
A
B
MINUTES SURVIVAL
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
104.0
112.0
116.0
120.0
EXPOSURE TEMPERATURES °C.

FIGURE 9

RELATION OF THERMAL RESISTANCE TO GROWTH AND EXPOSURE TEMPERATURES

CULTURE 4

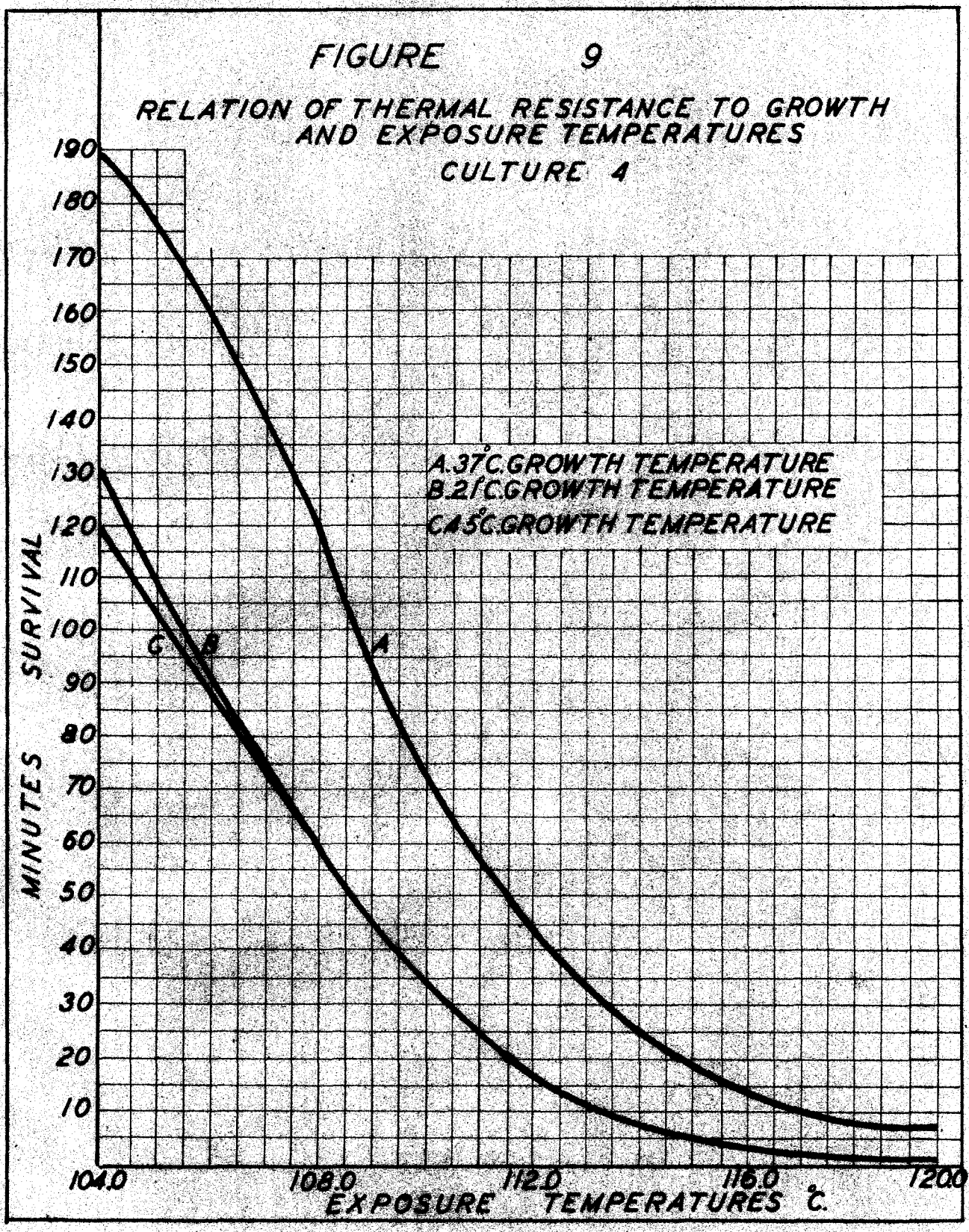


FIGURE 10

RELATION OF THERMAL RESISTANCE TO GROWTH AND EXPOSURE TEMPERATURE

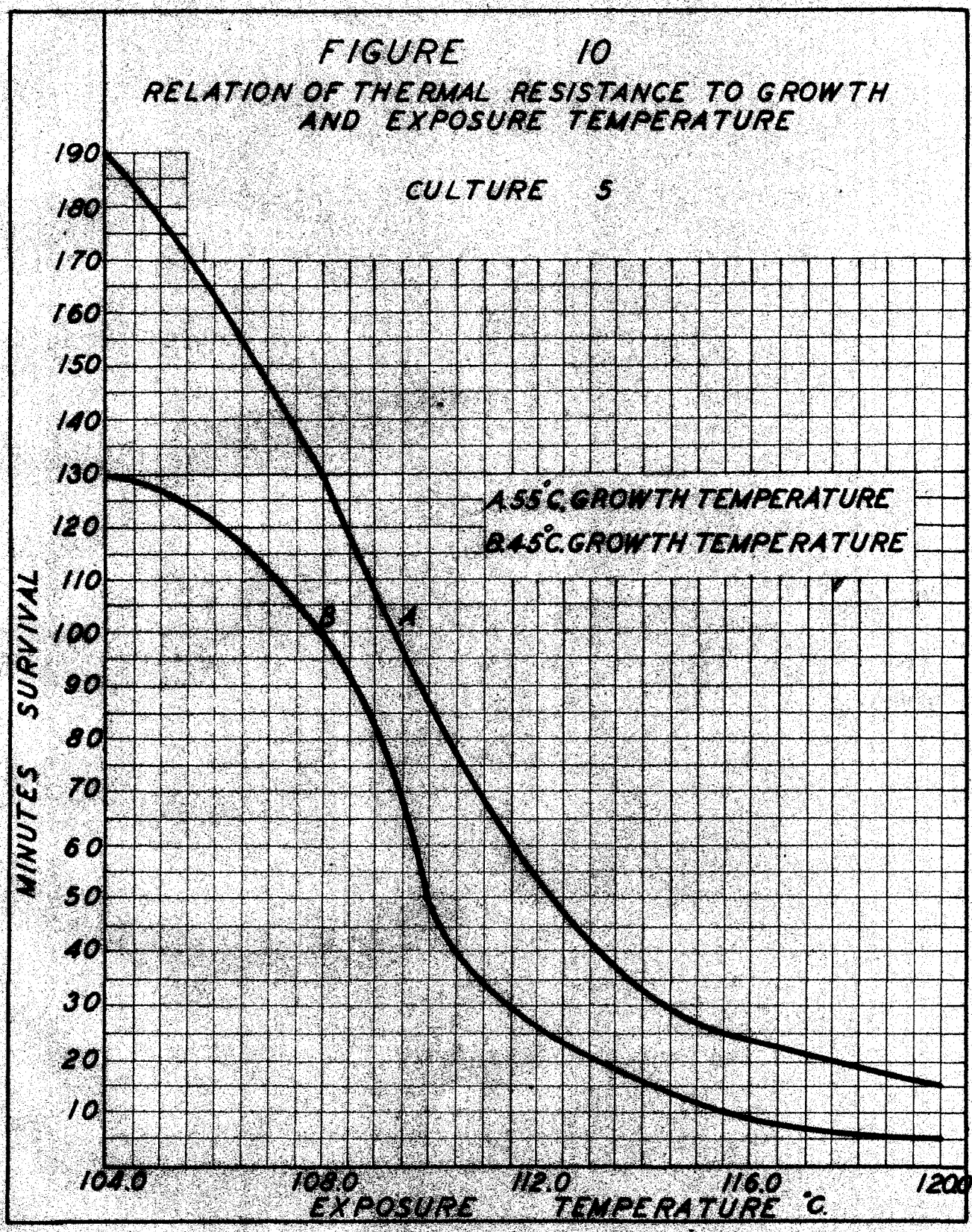


FIGURE 11

RELATION OF THERMAL RESISTANCE TO GROWTH AND EXPOSURE TEMPERATURES

CULTURE 9

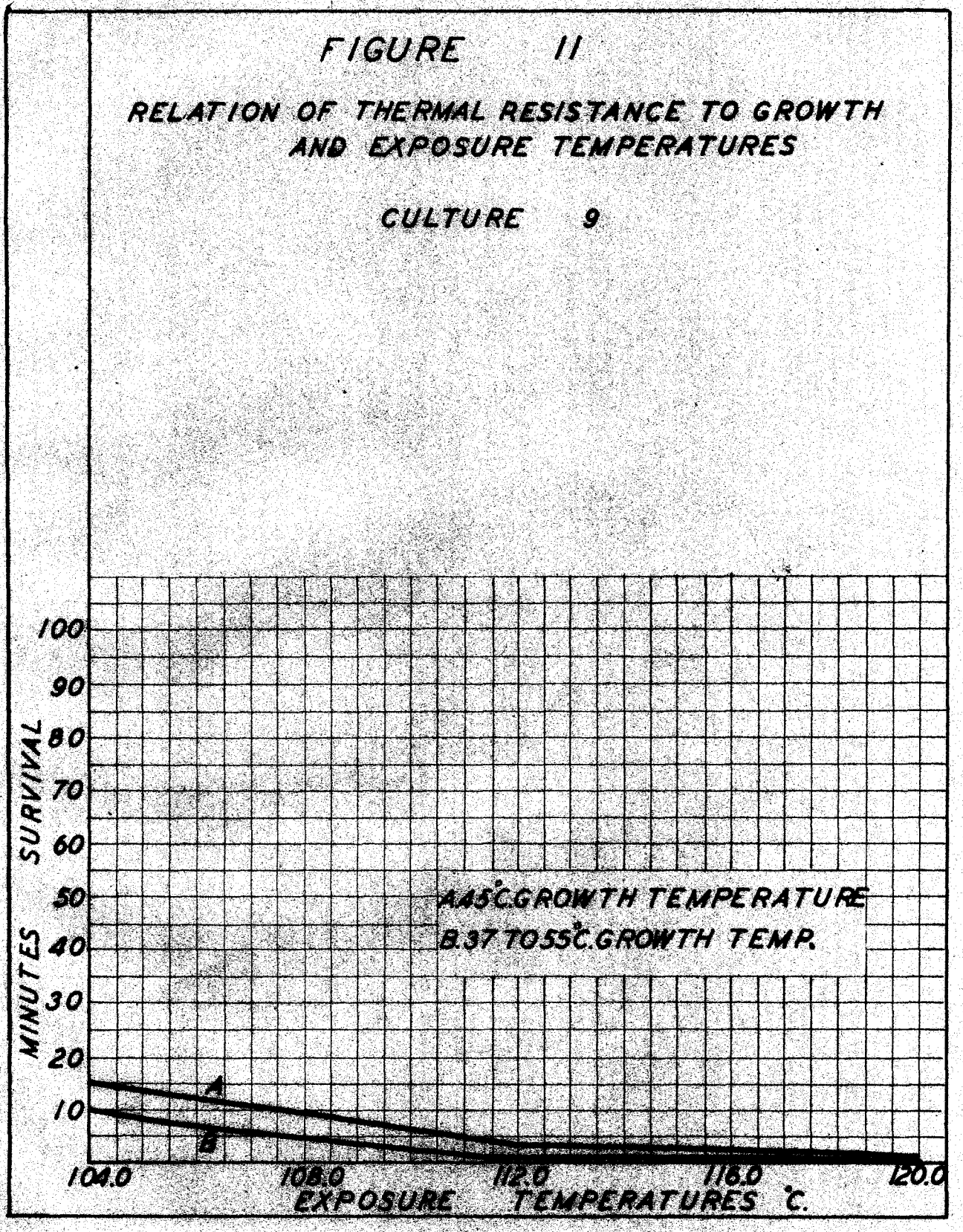


FIGURE 12

RELATION OF THERMAL RESISTENCE TO GROWTH AND EXPOSURE TEMPERATURE

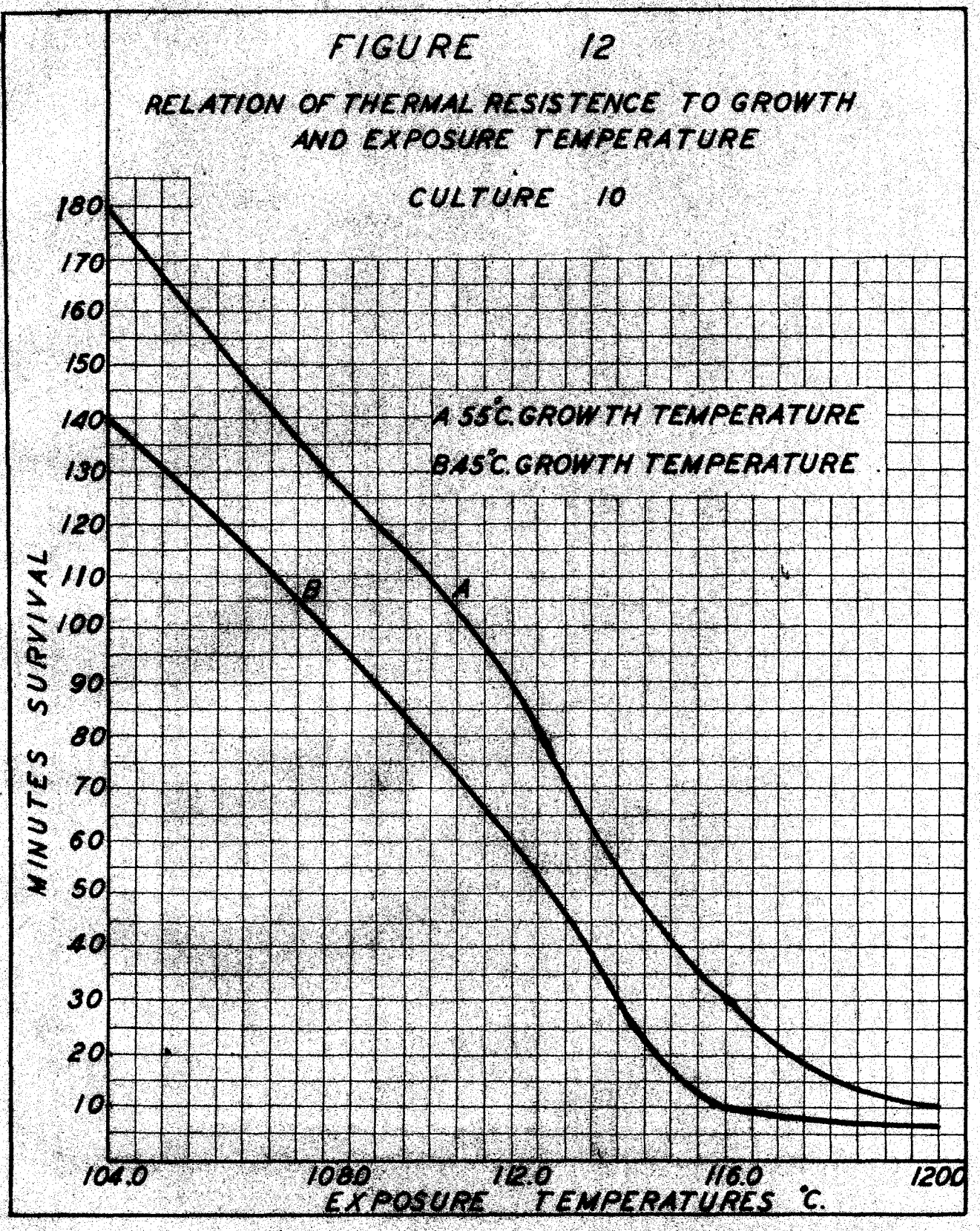
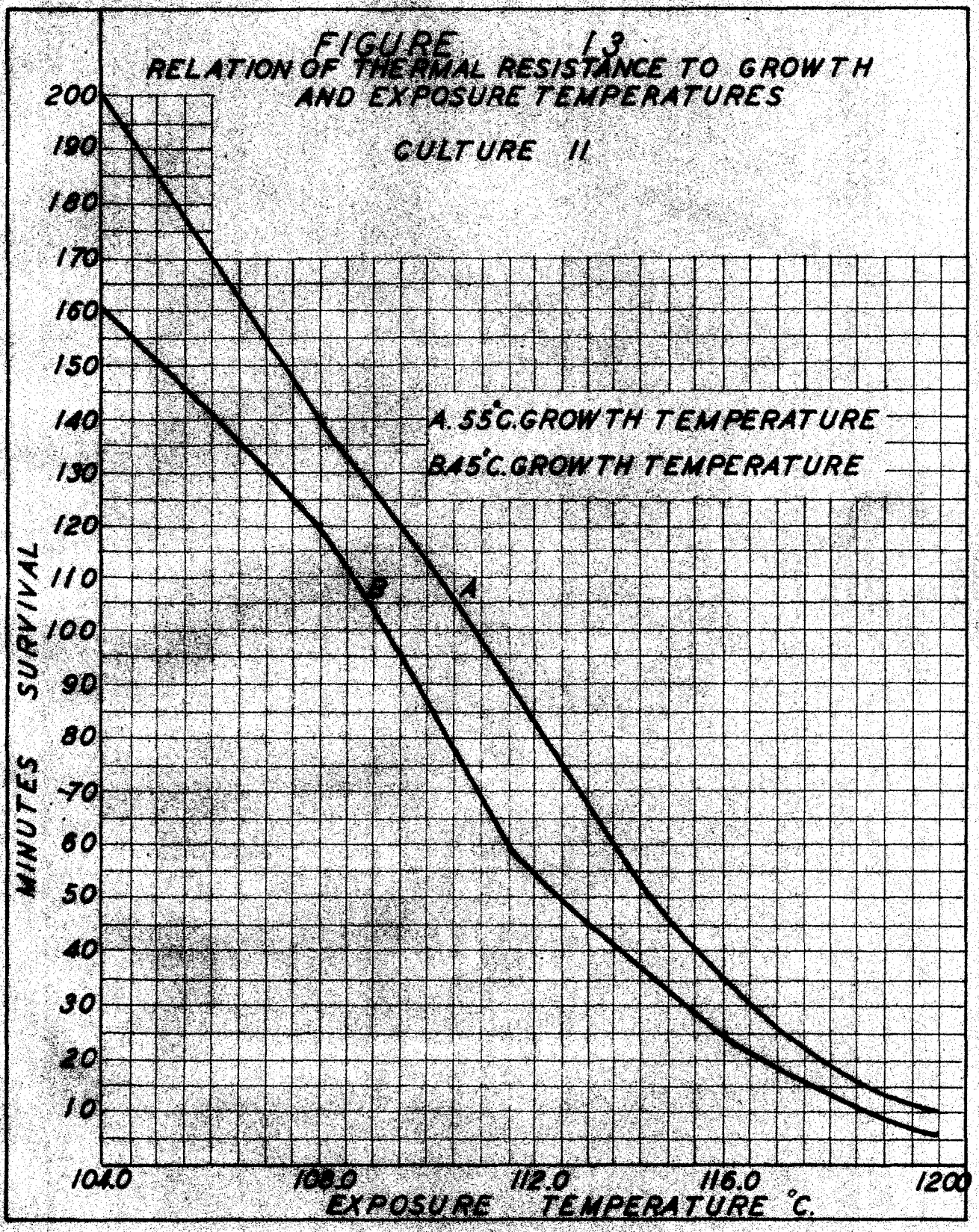


FIGURE 13

RELATION OF THERMAL RESISTANCE TO GROWTH AND EXPOSURE TEMPERATURES

CULTURE II



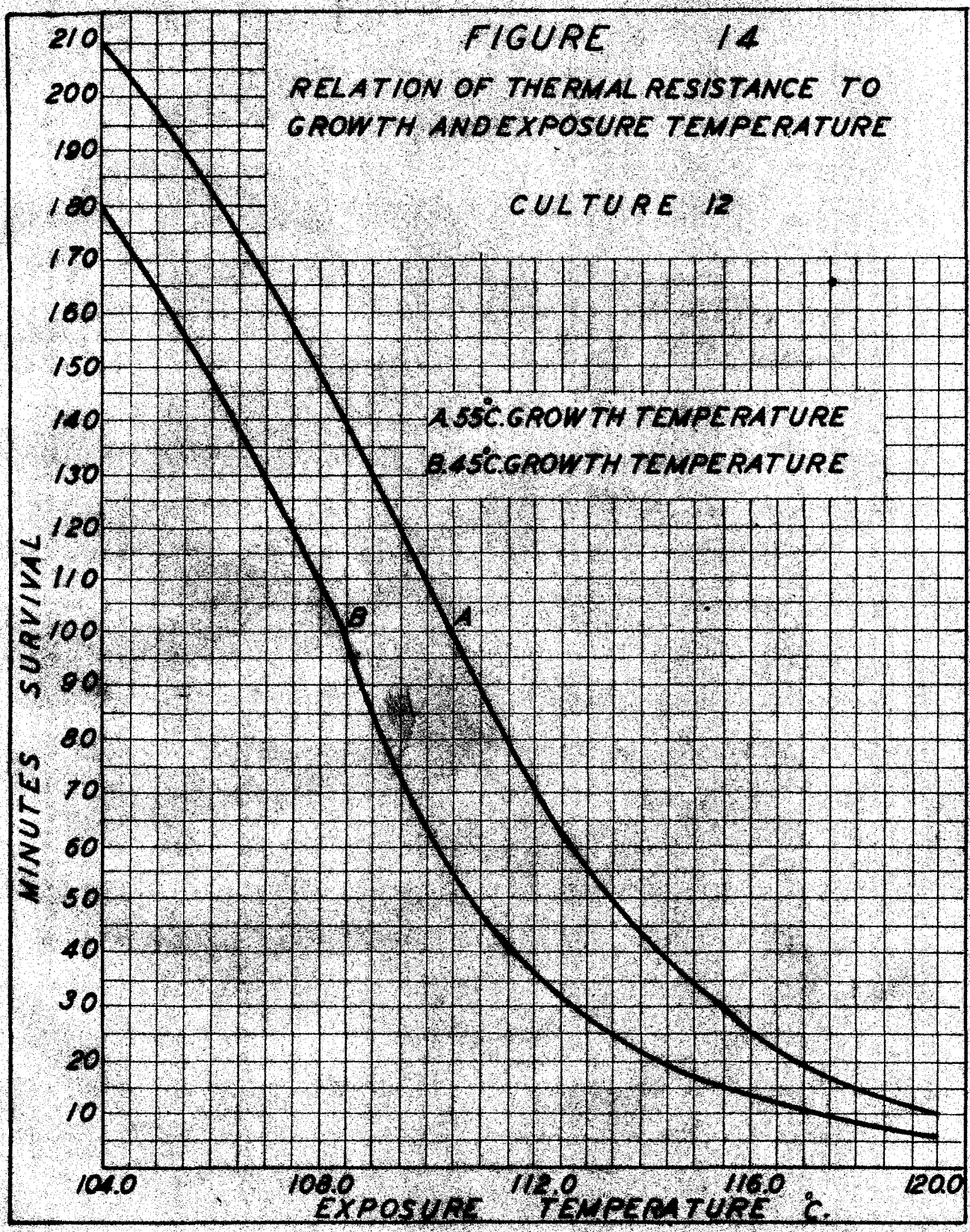


FIGURE 14
RELATION OF THERMAL RESISTANCE TO GROWTH AND EXPOSURE TEMPERATURE
CULTURE 12
A. 55°C. GROWTH TEMPERATURE
B. 45°C. GROWTH TEMPERATURE
MINUTES SURVIVAL
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
A
B
104.0
108.0
112.0
116.0
120.0
EXPOSURE TEMPERATURE °C.

There was considerable variation among the various cultures in the survival at the different exposure temperatures. The survival in all instances varied with the temperature of growth. Optimum growth temperatures always gave the greatest survival of the spores at each exposure temperature. A study of the data presented in Tables 4, 9, 11, and 12 show that the maximum thermal resistance of moist spores was secured by growth at the optimum temperature in 29 out of 32 comparative trials or 90.6 per cent of the trials. An analysis of the data presented in Tables 2, 4, 6, 8, 9, 11, and 12 also shows that growth temperatures below the optimum decreased the thermal resistance of moist spores in 100 of 104 comparative trials or 96.1 per cent of the trials. Using the growth at the optimum temperatures for comparision, the data in Table 12 show that the survival at an exposure temperature of 104.0°C. varied from 15 minutes with culture 9 to 210 minutes with culture 12. Cultures 1, 2, 4, 5, 10, and 11 survived for 160, 190, 190, 190, 180, and 200 minutes, respectively, at 104.0°C. The same order of survival held to a large extent with the exposure temperatures of 108.0°, 112.0°, and 116.0°C. At 120.0°C. the survival ranged from 1 minute with culture 9 to 15 minutes with culture 5. It is interesting to note that at 116.0°C., or the exposure temperature approximating that used in the sterilization of evaporated milk, a survival of 15 minutes or more was shown by cultures 1, 4, 5, 10, 11, and 12 which sur-

vived for 15, 15, 25, 30, 35, and 30 minutes, respectively. These observations substantiate data presented in Tables 2, 3, 4, and 5.

Lowering the exposure temperature would naturally increase the survival period with all cultures. Using an average of the survival periods of all the cultures for the various exposure temperatures, when grown at their optimum temperatures, it was found that lowering the exposure temperature from 120.0° to 112.0°C. increased the period of survival 7.7 times. Lowering the exposure temperature from 112.0° to 104.0°C. increased the period of survival 2.9 times. The results indicate that the rate of increase in length of survival associated with a decrease in exposure temperature tends to be much closer to a geometric than an arithmetic progression.

Examination of normal samples of evaporated milk

Fifteen samples of evaporated milk, representing seven different brands, were secured from retail stores and examined for bacteria. The presence of organisms was determined by transferring a small amount (about 0.1 cc.) of the evaporated milk to each of three beef infusion agar slants, incubating at 37°, 45°, or 55°C. for five days and examining for growth. Results of the examination are presented in Table 13.

Table 13

Examination of normal samples of evaporated milk

Sample Number	pH	Growth at 21°C.	37°C.	45°C.	55°C.
1	6.5	-	-	-	-
2	6.5	-	-	-	-
3	6.4	-	-	-	-
4	6.4	-	-	-	-
5	6.3	-	-	-	-
6	6.4	-	-	-	-
7	6.3	-	-	-	-
8	6.5	-	-	-	-
9	6.5	-	-	-	-
10	6.5	-	-	-	-
11	6.4	-	-	-	-
12	6.3	-	-	-	-
13	6.3	-	-	-	-
14	6.4	+	+	+	-
15	6.4	-	-	-	-

Only sample number 14 gave any evidence of bacterial contamination. Neither the flavor and odor nor the body of the evaporated milk was adversely affected by this contamination. Culture 17 was isolated from the milk. Spores of culture 17, grown at 21°, 37°, or 45°C. and heated in sterile skimmilk, were destroyed by a temperature as low as 104.0°C. in as short a period as 1 minute. The spore content of the skimmilk ranged from 100 to 200 per cubic centimeter. Apparently, this organism would have been easily destroyed by the ordinary sterilization procedure if it had been present when the milk was heated, so it seems probable that the contamination occurred after sterilization, although it is entirely possible that the spores originally present may have been very resistant for some reason.

If the results can be considered as representative, it appears that the presence of viable spores or bacterial cells in evaporated milk is uncommon.

Thermal resistance of some aerobic, spore-forming bacteria isolated from raw milk

Aerobic, spore-forming bacteria have been found repeatedly in raw milk, and some of these have exhibited rather high resistance to heat. The work of Lawrence and Ford (46) on this subject is outstanding. In order to determine the possibility of ordinary raw milk containing

aerobic, spore-forming bacteria especially resistant to heat, a limited number of samples were examined on two different occasions. It was hoped that the examination would give some indication of the frequency of especially heat resistant types of aerobic, spore-forming bacteria in raw milk. From such information at least some inference could be drawn as to the possibility of raw milk being the source of organisms surviving the sterilization process used in manufacturing evaporated milk.

Twelve samples of raw milk were heated at 80°C. for 10 minutes. The samples were then divided into two portions and one portion was enriched by incubating at 37°C. for 24 hours while the other portion was similarly enriched at 55°C. Following the enrichment processes the samples were plated on beef infusion and nutrient agar. Enough plates were poured so that four incubation temperatures could be used with plates from each portion of each sample. Plates were incubated at 21°, 37°, 45°, and 55°C. for from 24 to 48 hours. Typical colonies were then streaked on beef infusion agar and nutrient agar slants. Every sample of milk contained aerobic, spore-forming bacteria, as shown by growth on the agar plates incubated at one or more of the temperatures used. Many of the plates contained more than one type of colony (usually two), but in these instances a colony representing the type most numerous on the plate was picked. Some of the

plates, however, contained only one type of colony. Frequently the same type of colony was present on the plates incubated at different temperatures, and in these instances a colony from the plate showing the most luxuriant growth was picked. Cultures secured by this general procedure were designated as A, B, C, D, E, F, G, H, I, J, K, L, and M. Each of the cultures was from a different sample of milk except cultures L and M which were from the same sample.

Thermal resistance trials were conducted on all the cultures at 110.0°C. The trials were conducted in the same manner as those in the case of the cultures isolated from evaporated milk. The spore contents of the spore suspensions varied from 1,000 to 10,000 per cubic centimeter with the different cultures. Table 14 presents the results obtained. From the data it is evident that none of the cultures showed a thermal resistance which would remotely suggest a possible survival of the usual sterilization procedure employed in manufacturing evaporated milk. The greatest thermal resistance exhibited by the spores was 3 minutes survival at 110.0°C. and this occurred with cultures F and G grown at 37°C. and with cultures H and I grown at 55°C.

Although the number of samples of raw milk examined was limited, the results indicate that raw milk commonly contains aerobic, spore-forming bacteria. These organisms, however, apparently do not usually possess a very high thermal resistance. Consequently, raw milk should not ordinarily be

Table 14

Thermal resistance of cultures isolated from raw milk

Culture	Growth at				*Minutes survival when grown at			
	21°	37°	45°	55°	21°	37°	45°	55°
A	+	+	+	-	0	0	0	-
B	-	-	-	+	-	-	-	0
C	-	+	+	+	-	0	0	0
D	-	+	+	-	-	0	0	-
E	+	+	+	-	0	0	0	-
F	-	+	+	+	-	3	0	0
G	-	+	+	+	-	3	0	0
H	-	-	+	+	-	-	0	3
I	-	-	+	+	-	-	0	3
J	-	-	+	+	-	-	0	0
K	-	-	+	+	-	-	0	0
L	-	-	+	+	-	-	0	0
M	-	-	+	+	-	-	0	0

* 110.0°C. Exposure temperature.

considered a common source of bacteria capable of surviving the usual sterilization procedure followed in the manufacture of evaporated milk although, presumably, it may be a source under certain conditions.

Identification of the bacteria used in the study

A total of 28 cultures was used in the study. Some of the cultures isolated, notably cultures 13, 15, 16, and 17, did not exhibit any appreciable resistance to heat and, consequently, were not used in any of the heat resistance trials.

In order to make possible the comparison of the results with other published work, an attempt was made to identify the various cultures. Cultures 6 and 14 were not identified as they were found to be cocci, while cultures H, I, J, K, L, and M were not identified as they exhibited very little resistance to heat and are evidently unimportant in evaporated milk. Using "Bergey's Manual of Determinative Bacteriology" (6) as a guide, the cultures were identified as follows:

Culture 1 - Bacillus megatherium de Bary

Culture 2 - Bacillus freudenreichii (Miquel) Migula

Culture 3 - Bacillus albolactis Migula

Culture 4 - Bacillus lactimorbus Jordan and Harris

Culture 5 - Bacillus calidolactis Hussong and Hammer

Culture 7 - Bacillus petasites Gottheil

Culture 8 - Bacillus cereus Frankland and Frankland

Culture 9 - Bacillus coagulans Hammer

Culture 10 - Not identified

Culture 11 - Not identified

Culture 12 - Bacillus calidolactis Hussong and Hammer

Culture 13 - Bacillus lactis Flügge

Culture 15 - Bacillus mesentericus Trevisan

Culture 16 - Bacillus vulgatus Trevisan

Culture 17 - Bacillus sphaericus Neide

Culture A - Bacillus mesentericus Trevisan

Culture B - Bacillus kaustophilus Prickett

Culture C - Bacillus mycoides Flügge

Culture D - Bacillus mycoides Flügge

Culture E - Bacillus megatherium De Bary

Culture F - Bacillus cereus Frankland and Frankland

Culture G - Bacillus subtilis Cohn emend. Prazmowski

Cultures 10 and 11 were not identified as they did not fit the description of any organism listed in Bergey's Manual (6).

It is interesting to note that several of the organisms identified were present in both raw and evaporated milk, namely, Bacillus megatherium, Bacillus cereus, and Bacillus mesentericus. Bacillus megatherium was the only one of these three organisms that was isolated from spoiled evaporated milk. The strain of Bacillus megatherium isolated from spoiled evapo-

rated milk exhibited a fairly high resistance to heat, while the strain from raw milk did not show any especial resistance to high temperatures. Bacillus megatherium isolated from spoiled evaporated milk, when grown at 21°C., showed a survival of 60 minutes at 108.0°C., 30 minutes at 112.0°C., and 5 minutes at 116.0°C. The same culture, when grown at 37°C., showed a survival of 80 minutes at 108.0°C., 40 minutes at 112.0°C., and 15 minutes at 116.0°C. In contrast, the strain of Bacillus megatherium isolated from raw milk, when grown at either 21° or 37° C., did not survive even one minute at 110.0°C. The much greater thermal resistance of the strain of Bacillus megatherium isolated from spoiled evaporated milk is significant and agrees with the work of Kelly (45). The only explanation which can be given for the greater thermal resistance is that higher resistance had been acquired through natural selection and acclimatization of the strain.

The strain of Bacillus cereus isolated from evaporated milk possessed approximately the same thermal resistance as the strain isolated from raw milk. The strain from evaporated milk survived 3 minutes at 103.0°C. when grown at 37°C., and the strain from raw milk survived 3 minutes at 110.0°C. when grown at 37°C. Neither the strain of Bacillus mesentericus isolated from evaporated milk nor the strain isolated from raw milk, survived an exposure of 110.0°C. for even one minute, when grown at 21°, 37°, or 45°C.

DISCUSSION OF RESULTS

The importance of low temperatures for holding milk which is to be manufactured into evaporated milk is indicated by the data showing the relatively low thermal resistance of aerobic, spore-forming organisms grown at temperatures below the optimum. It appears, therefore, that the maintenance of low temperatures would markedly lower the thermal resistance of bacteria likely to be present at the time of sterilization and thereby reduce, if not prevent, the spoilage of evaporated milk. An exception to the beneficial influence of low temperatures would be in the case of using temperatures low enough to inhibit the growth of spoilage organisms but still having present in the milk spores which had developed at high or optimum temperatures. In this instance the maintenance of low temperatures would perhaps not markedly lower the thermal resistance of the spores. The observation emphasizes the necessity of maintaining low temperature, wherever possible, from the time the milk is drawn until the time of sterilization. The data presented give support to the observations of Weil (87) that growth temperatures influence the ability of microorganisms to resist heat.

The results show that the temperature to be avoided in attempting to secure a lower thermal resistance of spores of organisms isolated from evaporated milk is the optimum

growth temperature. Although growth temperatures above the optimum tend to reduce the heat resistance of the spores, such holding temperatures are objectionable since some of the organisms found in evaporated milk have a high optimum growth temperature, and high temperatures (37° to 55°C.) adversely affect the quality of the milk due to the development of bacteria which may lower the heat stability of the evaporated milk. Realization of the great thermal resistance exhibited by some of the aerobic, spore-forming bacteria gives a better appreciation of the possibility of milk spoilage even when employing a sterilizing procedure that is usually efficacious. In fact, culture 5, at its optimum growth temperature, produced spores that could survive the ordinary autoclaving process used in laboratory work of 120.0°C. for 20 minutes.

The observations indicating that each growth temperature may give a different plane of heat resistance to the spores of specific organisms are of interest. From the commercial viewpoint they emphasize the necessity of continually maintaining temperatures which are unfavorable for growth. The data presented show that growth of organisms at favorable temperatures for only one generation resulted in a material increase in the heat resistance of the spores. The observations, therefore, form a basis for an explanation of the sudden outbreaks of spoilage in evaporated milk. Briefly, (assuming the presence of causal organisms on the farm or in

the plant) the prevalence of a favorable growth temperature for several days (sufficiently long to produce spores) might increase the heat resistance of the spores enough so that if they should gain entrance into the milk they would survive the usual heat treatment given the evaporated milk.

The relative constancy of the results obtained in the various heat resistance trials with each culture, as long as the growth temperature was the same, is in harmony with the work of Morrison and Rettgers (53) but is not in agreement with the observations of Esty and Williams (29) and Magoon (49). The latter investigators consider the resistance of spores to heat as not a fixed property but a variable characteristic, influenced by a host of conditions rather than by one factor such as temperature of growth.

The tendency for moist spores to have a greater thermal resistance than either freshly dried (5 to 8 days old) or aged dried spores (47 to 69 days old) is in agreement with the work of Esty and Meyer (28). The practical aspect of this observation is that although fresh, moist spores may be more resistant to heat, dried spores may well be the cause of spoilage outbreaks in evaporated milk if they happen to get into the milk from a source in the plant. Any control measures inaugurated must, therefore, include the elimination or destruction of dried spores in the plant.

The tendency of spores from cultures grown for ex-

tended periods on artificial culture media to decrease in their resistance to heat is in general agreement with the findings of Curran (19). The relationship between thermal resistance and growth on artificial culture media was influenced not by the numbers of transfers made but rather by the total time the cultures were carried on the media. If continued growth of organisms on artificial culture media decreases the thermal resistance of the spores produced, it naturally follows that spores should possess their maximum thermal resistance when produced in the natural habitat of the organism. It should, therefore, be recognized that with the cultures studied, natural conditions of habitat are probably conducive to maximum thermal resistance. From a practical viewpoint these results emphasize the necessity of protecting the milk from contamination on the farms of the producers, since it is generally true that most of the organisms present in milk are present as the result of improper production methods. Contamination on the farms undoubtedly would be with spores or cells possessing their maximum thermal resistance for the specific growth temperature.

Results indicating that, in general, the survival of the spores varied inversely with the exposure temperature are in agreement with the work of Bigelow and Esty (8).

The isolation of viable cells or spores from only

one of 15 representative samples of normal evaporated milk suggests that the presence of organisms is not common. Although spoilage was not evident in this instance, it signifies an opportunity for spoilage and the need for a better sterilization procedure or closer control throughout the entire manufacturing process. It is entirely possible that the causal organism may have gained entrance to the evaporated milk after the sterilization process because of a defect in the tin.

Although no aerobic, spore-forming organisms exhibiting any marked resistance to heat were isolated from raw milk, the presence (in three instances) of the same species of bacteria in both raw and evaporated milk is of interest. This observation, together with the work of Hammer and Hussong (41), Kelly (45), and Morrison and Rettger (53), who reported instances of spoilage of evaporated milk caused by aerobic, spore-forming bacteria commonly found in raw milk, shows that raw milk must always be considered as a potential source of contamination.

The greater thermal resistance of the culture of Bacillus megatherium found in spoiled evaporated milk than of the culture found in raw milk can be explained only by considering them as two different strains, with the strain from evaporated milk possessing a greater thermal resistance as the result of natural selection and acclimatization.

The presence of Bacillus freudenreichii, Bacillus albolactis, Bacillus lactimorbus, Bacillus petasites, Bacillus sphaericus, and Bacillus lactis in evaporated milk has not been previously reported. None of these organisms caused spoilage. All organisms isolated from raw milk have been reported by several investigators, notably Lawrence and Ford (46) and Prickett (56), as having been found in milk.

SUMMARY AND CONCLUSIONS

The work reported involved a study of the influence of growth temperature on the thermal resistance of spores of certain aerobic, spore-forming bacteria isolated from normal and spoiled evaporated milk. Within the limits of the study, as imposed by the number and species of organisms used, the following points were established:

1. The thermal resistance of spores was influenced by the temperature at which the cultures were grown.

2. Growth temperatures below the optimum decreased the thermal resistance of the spores in 96.1 per cent of the trials.

3. Growth temperatures above the optimum tended to decrease the thermal resistance of the spores and the decreases were generally as great as that caused by growth at temperatures below the optimum.

4. Maximum thermal resistance of the spores was obtained by growth at the optimum temperature in 90.6 per cent of the trials.

5. Some cultures isolated from evaporated milk, grown at the optimum temperature, produced spores which, when present in large numbers, survived the sterilization exposure normally used in manufacturing evaporated milk. This was true of cultures 1, 2, 4, 5, 10, 11, and 12.

6. Sudden decreases in growth temperature from the optimum always decreased the average thermal resistance of the spores, while sudden increases in growth temperature to the optimum always increased the thermal resistance, thus establishing the important influence of growth temperature on thermal resistance.

7. Continued growth of cultures at changed growth temperatures generally resulted in spores with a thermal resistance approximating that of spores of the cultures when originally grown and tested at the same temperatures.

8. Moist spores tended to have a greater thermal resistance than either freshly dried spores (5 to 8 days old) or aged dried spores (47 to 69 days old).

9. The influence of age on the heat resistance of dried spores was variable, but the tendency was for the aged spores to be more heat resistant than the freshly dried spores. The influence of age was not in any manner correlated with the growth temperature of the spores.

10. Prolonged periods of growth on artificial culture media tended to decrease the thermal resistance of the moist spores of the cultures studied. Any relationship existing between the thermal resistance and growth on artificial culture media apparently involved the length of time the cultures were carried on the media and not the number of transfers made in carrying the cultures.

11. The thermal resistance of the spores varied inversely as some function of the temperature of exposure, and the rate of increase in the time of survival with a decrease in exposure temperature tended to be much closer to a geometric than an arithmetic progression.

12. Viable spores or cells of bacteria were not common in the normal evaporated milk examined.

13. From the 12 samples of raw milk studied no aerobic, spore-forming bacteria possessing an especially high thermal resistance were obtained, but raw milk should always be considered as a potential source of contamination of evaporated milk with heat resistant bacteria.

14. The strain of Bacillus megatherium isolated from spoiled evaporated milk exhibited a high thermal resistance, while the strain of Bacillus megatherium isolated from raw milk did not.

15. Bacillus megatherium, Bacillus calidolactis, and Bacillus coagulans were found present in spoiled evaporated milk.

16. Bacillus freudenreichii, Bacillus albolactis, Bacillus lactimorbus, Bacillus petasites, Bacillus cereus, Bacillus lactis, Bacillus mesentericus, Bacillus vulgatus, and Bacillus sphaericus were found present in normal evaporated milk.

17. Bacillus mesentericus, Bacillus kaustophilus, Bacillus

mycoides, *Bacillus megatherium*, *Bacillus cereus*, and *Bacillus subtilis* were isolated from raw milk.

18. The results of the study suggest that in order to decrease the thermal resistance of bacteria likely to be found in evaporated milk and thus minimize or prevent spoilage losses, low temperatures should be maintained in the raw milk from the time of production until it reaches the forewarmer or preheater. Likewise, during any storage period previous to the sterilization process, low temperatures should be maintained. Further, any "clean up" system inaugurated should include methods for the destruction of dried spores present in the plant and on the equipment.

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