FACTORS IN THE EXTENSION OF SHELF LIFE OF RATION COMPONENTS

by

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FACTORS IN THE EXTENSION OF SHELF LIFE OF RATION COMPONENTS by LAWRENCE SANFORD SPIEGEL. Submitted to the Department of Food Technology on 13 January 1958 in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

This work was designed to explore possible methods of processing "snack" meal items to eliminate the hazard of food poisoning by enterotoxin producing Micrococcus pyogenes, var. aureus. Military feeding situations were used as a guide in such areas because there frequently arises the necessity for large-scaled preparation of menus some time prior to consumption. Without refrigeration between fabrication and eating, food poisoning is not an unlikely consequence. There are many comparable civilian situations.

The investigator studied some of the fundamental problems of pasteurization using sandwich components as an experimental basis because of the many problems concerned with the handling of these types of food materials. The fillings used were boiled ham and roast beef slices. These were shown to have relatively high resistance to quality changes when subjected to the various processing operations, and are also high acceptance foods. The work had a duality of phases, the nature of the lethal response of Micrococcus pyogenes, var. aureus to heat, and a study of simulated commercial operations, using food materials having a high initial contamination of the indicator organism.

Measurement of the thermal death rates were made in a temperature range of 125° to 200°F. Between 125° and 140°F, the slope of the thermal death time curve was 11.3°F, a value in agreement with published results. However, above 140° to 200°F, the heat requirements of the organism increased in terms of time and temperature, altering the slope and the linearity of the thermal death time curve.

A number of chemical substances, many of which are permitted in foods, and four antibiotics were evaluated for their effect on the thermal resistance of Micrococcus pyogenes, var. aureus. Significant changes were found with low concentrations of Aureomycin, Terramycin, subtilin, and nisin and several chemicals and chemical combinations.

Methods of packaging meat slices were developed to provide maximum protection during processing by heat and cathode ray irradiation. Inoculated slices, with residual flora, were processed under simulated commercial conditions. The effectiveness of different treatments was measured by bacteriological methods. The rate of reduction and the times required to reduce the microbial flora by 90% (D value) and 99.99999% (assumed end point) were found for air oven heating at 185° to 300°F and irradiation doses of 100 to 1500 x 10^3 rep. It was found that heating and irradiation might be suitable for applications as industrial processing operations for these foods.

Processed slices were also frozen. Freezing both heated and irradiated slices at 0°F for 24 hours resulted in approximately a 60% reduction due to freezing.

Individual bread slices were shown to have a very rapid rate of staling. Preservation of quality by freezing could only be done when the slices were packed at high vacuum. A method was developed for creating these vacuum conditions within a film package. The problem was the collapse of crumb cells with the rapid removal of air. This was overcome by freezing the slices in an unsealed package for crumb rigidity, applying the vacuum, and heat-sealing while the slice was still in the frozen state. This procedure produced a remarkable improvement over air or partial vacuum-packed slices.

It is possible that the results of this work may be used to elaborate methods which could be applied to process snack foods, properly store them until use, and then hold them for periods of up to one day, or even longer, at ambient temperatures above freezing without danger of causing food poisoning.

Thesis Supervisor: Professor Bernard E. Proctor

Title: Head, Department of Food Technology

Salem, Massachusetts

13 January 1958

Professor L. F. Hamilton
Secretary of the Faculty
Mass. Institute of Technology
Cambridge, Massachusetts

Dear Sir:

Herewith is my thesis entitled, "Factors in the Extension of Shelf Life of Ration Components", submitted to the Department of Food Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Respectfully submitted,

Signature redacted

Lawrence Sanford Spiegel

TO

ANNA AND THE LATE CHARLES SPIEGEL

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I.

INTRODUCTION

I. INTRODUCTION

The background of this work, as indicated by the title, is of a military nature. However, the general applications of the work are of a broader nature. Military operations are frequently dependent upon feeding large numbers of rations at a considerable distance from the point of preparation, with no intervening refrigeration. These two conditions prevail within the military, but are also met frequently in civilian situations.

This work was based specifically on Air Force experiences with the preparation of foods for in-flight feeding. Kitchens are maintained 24 hours each day at all flight bases for the preparation of perishable meals to be consumed during flight operations. These foods have a prescribed life of five hours, within which time they must be consumed. This limit of five hours was set by the Air Force, using as its basis, the time thought to be required by Micrococcus pyogenes, var. aureus to grow and produce enterotoxin in foods. The Air Force maintains these kitchens to prepare perishable food menus because these foods have high acceptability with crewmen, and the attendant morale value is important. These foods are also convenient to prepare and eat.

The concern about staphylococcal food poisoning arises from the almost immediate onset of symptoms after ingestion of toxins formed by bacteria. This is because the toxic agent is an enterotoxin, and therefore, unlike infectious diseases, no time is required for the transport of the infecting organisms to the loci of growth and for subsequent growth during which symtoms gradually develop. Although the effects of this type of food poisoning are generally not too serious, nor of lasting duration, they do cause severe gastro-enterological duress, which, during its course of about one day, is crippling. This demobilization could render a crew unfit to perform the most routine flying operations.

This thesis involves the investigations of possible methods by which the hazard of poisoning due to Micrococcus pyogenes, var. aureus may be eliminated from perishable, sandwich-type foods. Foods in sandwich form offer a broad base of components and problems.

Physical and chemical deterioration of the individual components are greatly accelerated when foods are prepared in sandwich form. Bread becomes dry and stales very rapidly when slices are exposed to air, and thin fillings dry quickly losing their moisture to the bread. The complexity of this type of food is further concerned with certain public health aspects. The fabrication of sandwiches requires a great amount of handling of each component. Superimposed upon the

usual microbiological problems based on the indigenous flora and particular characteristics of each component is the added danger of microbiological contamination from the handler.

The approach to the development of processing techniques, through which means, bacterial populations could be reduced to negligible levels without degrading the quality of the foods, is in two parts.

The first is a basic study of the sensitivity of Micrococcus pyogenes, var. aureus to various lethal agents. This fundamental work is important in understanding and defining the conditions influencing the rates of lethality.

The second phase is concerned with the application of various processing techniques and the development of suitable packaging methods for the pasteurization of these foods under conditions compatible with the maintenance of high organoleptic quality.

A high degree of pasteurization was sought rather than sterilization, for several reasons. Firstly, these foods are designed for eating within a few hours; an extension of shelf life to a limit of one day would be of considerable value. Pasteurization may provide this extension. Beyond this time limit, sterilization would be required. Secondly, processing designed to achieve pasteurization, being a less severe process and thus less destructive to foods than sterilization, offers greater possibilities for

finding methods that have immediate application. Finally, the factor most likely to predispose staphylococcal poisoning is the contamination of foods with this organism during the handling of sandwiches. Because Micrococcus pyogenes, var. aureus is a vegetative type (non spore-forming), pasteurization can be expected to reduce this organism to negligible levels. The value of sterilization would be in the further destruction of bacterial spores, which is beyond the scope of the work covered by this thesis.

The isolation and study of the problems associated with individual areas of the problem as outlined above, the development of bases for tentative solutions, and derived from this work, suggestive programming for possible commercial operations, are included.

II.

LITERATURE SURVEY

II. LITERATURE SURVEY

A. MICROCOCCUS PYOGENES, VAR. AUREUS

1. Food Intoxication

Micrococcus pyogenes var. aureus is responsible for the most common type of food poisoning. This poisoning is known as staphylococcal poisoning, and is caused by toxin production within the food, which results in a rapid onset of symptoms after ingestion. Although these symptoms are quite severe, recovery is usually complete within a few days. This type of food poisoning is usually incorrectly described by the layman as "ptomaine poisoning".

It is believed that only a fraction of the cases are reported to public health authorities. The cases which usually are reported are those in which large numbers of people are affected. For example, one of the organisms used in this work was isolated from a group of boys at a summer camp in Massachusetts. The source of the organism was apparently ham, fed in sandwiches to all of the campers, most of whom were afflicted and required hospitalization. Isolated cases are seldom reported.

This intoxication is reviewed in detail by Dack (1943), Tanner (1945) and Jensen (1957). A fascinating, if somewhat informal, account of the enterotoxin effects was described by Dolman (cited by Jensen, 1957):

> "The vomiting is accompanied, and sometimes replaced by, considerable retching. At first one is astonished to recall portions of meals long forgotten. The ordeal can perhaps be compared to pregnancy, in that it may have been light-heartedly enough embarked upon the first time, but is not so willingly repeated; and in its being made bearable, as the situation moves towards a climax, only in the reflection that deliverance is bound to come ... Once begun, recovery was usually rapid, and the only after-effects being a large appetite, and a sense as of calm after a storm."

The feeding of military groups necessitates a freedom from danger of micrococcal poisoning. The possibility of losing the use of a group performing a vital task must be minimized. The Air Force is particularly concerned about this problem because inactivation of a flying crew would be likely to mean the loss of the aircraft. The probability of incidence of staphylococcal food poisoning is great with the Air Force because of the large number of flights routinely flown, and the necessity of supplying these flight crews with non-refrigerated food, certain to have been subjected to a considerable period out of refrigeration between the time of preparation and that of consumption. Wright Air Development Center Technical Report 54-354 (1954) completely surveys the feeding regimes

of flight menus. The flight feeding program is also discussed by Robertson (1956), Taylor (1956), Finkelstein (1956), and the Research and Development Associates, Quartermaster Food and Container Institute (1956).

Dyme (1957), describing the current feeding program, spoke of the high acceptability of snack-type meals, which are more economical than processed foods despite the daily maintenance of 24-hour kitchens at each flying activity to provide fresh menus to flying crews. The limitations governing the feeding of such items lies in the possible growth of food poisoning organisms in these foods. Micrococcus is the organism chiefly responsible for concern because of its frequent occurrence and because of the rapid inactivation of afflicted persons.

2. Occurrence

Micrococci causing food poisoning find wide distri-

bution in foods. A list by Feig (1944) of the principal food sources, based on the number of reported outbreaks, is as follows:

Meat
Poultry Products
Bakery Products
Milk Products
Vegetables

Segalove, Davison and Dack (1943) have shown that the growth of a food poisoning strain of Micrococcus pyogenes, var. aureus is not necessarily a property of the food itself, but rather, of its acidity. Growth occurs maximally in low acid foods.

Of special interest to this work is the presence of micrococcal cells causing staphylococcal food poisoning in meat products. Slocum and Linden (1939) reported that cooked hams and tongues caused 20 outbreaks of micrococcal poisoning, affecting over one thousand people. Other work concerning meat is reported: Dack and Surgalla (1953), Griswold (1950), Ingram (1955), Jensen (1954), McDivitt and Hussemann (1957), Scott (1955), Segalove and Dack (1951), and Surgalla and Dack (1945).

The organism has wide distribution in meat products. Fresh meat will frequently harbor the organism. Jepsen (1947) found Micrococci species and Achromobacter in high concentrations on hog skins. He described the presence of these genera as being perhaps as characteristic of meat as Streptococci are characteristic of milk.

Reith (1929) considered that the contamination of freshly slaughtered meat was due to invasion from the nasal passages and skin. Tanner (1944) presented arguments concerned with this explanation and generally described the flora of fresh meat, reported by a number of investigators, with a high percentage reporting the presence of Micrococci.

Cured meat products generally contain a greater number of Micrococci than fresh meats. Micrococci are commonly found in these products because of the natural contamination of the meat, the presence of these organisms in curing solution, and their salt tolerant nature. These, compounded with the contamination from handling operations, all tend to increase micrococcal populations. The fact that the curing solution will inhibit the growth of many other types of organisms, allows Micrococci and other halophiles to grow non-competitively. This situation was described by Garrard and Lochhead (1939).

Though this list encompasses a wide range, certain types of food are more likely, based on technique and handling during preparation, to cause staphylococcal poisoning than others. For example, within the bakery products group, bread and rolls are never mentioned as the cause of food intoxications, whereas custard pies, cream-filled doughnuts, chocolate and cream-filled eclairs and tarts

are often involved. (Kelly and Dack, 1936). These products have enough moisture for growth of the causative organism, come into contact with human hands, with the danger of contamination, and if baked, during the mild heating, growth is enhanced. These products are often held at higher temperatures than would slow down the growth of Micrococci, and thus growth may proceed to large numbers. Micrococci grow in media with a moisture content greater than 40 percent. (Segalove and Dack, 1951).

The distribution of Micrococci in nature is also very widespread, which partly accounts for its presence in food materials. The other major contribution of micrococcal cells to foods is brought about by handling operations. Jensen (1957) described their presence in the nose and throat, being carried in sneezing, coughs, and nasal discharges; boils and pimples; and infected cuts on hands. Dust also may harbor these cells.

The possible result of all of this is illustrated by Dack (1956):

> "An example *** involved 10 hams which were cooked until the internal temperatures reached 165°F, after which they were sliced and handled by a butcher who had severe facial acne. The sliced hams were covered with aluminum foil and kept at a warm temperature for 7-10 hours before serving *** those eating 7-10 hours later became acutely ill. The strain from the acne lesion and that recovered from the sliced ham were found by bacteriophage typing identical."

3. Enterotoxin

Dack (1943) and Bergdoll (1956) have established the basic chemical nature of the enterotoxin. It is a water soluble protein with a molecular weight of 24,000. Sixteen amino acids have been identified in the molecule, with lysine, tyrosine, aspartic acid and glutamic acid accounting for half of the concentration.

Two aspects of temperature which are important for the purpose of evaluating processing methods in terms of enterotoxin-producing Micrococci are interesting. Jensen (1957) showed that the temperature range for toxin production by Micrococci was 60° to 115°F, with optimum formation occurring between 90° to 97°F. Segalove and Dack (1951) and Gross and Vinton (1947) could not detect the production of enterotoxin at 43° and 44° F during a period of up to four weeks although all other conditions for such production were met. This work was carried out with meat products. These results indicate the importance of refrigeration in preventing the development of micrococcal toxin in foods in addition, of course, to the suppression of cellular growth at low temperatures.

The other aspect is the heat tolerance of micrococcal enterotoxin. Jordon, Dack, and Woolpert (1931) and Dack and Surgalla (1953) have found the toxin to be very highly heat resistant, and not destroyed in normal cooking pro-

cedures. This type of work is difficult to evaluate quantitatively because of the problem of measuring the toxin. The best test organism, unfortunately, is the human, and even the human will vary in reaction to micrococcal enterotoxin. The necessity to prohibit, by proper handling techniques, the growth of the large numbers of Micrococci required to produce enterotoxin is clearly indicated. Any heat processing operation must include sufficient treatment to provide a safety factor for this destruction, which may require 15 to 30 minutes at boiling water temperature. Although not all strains of Micrococcus pyogenes, var. aureus produce enterotoxin, it is wise to suspect any indication of this organism in food products.

4. Resistance

The degree of the resistance of Micrococcus pyogenes, var. aureus is very high among the nonsporulating bacteria. Zinsser (1948) lists some of the lethal chemical agents to which this species show resistance, and also show survival of viability during extreme conditions of drying.

The Phenol Coefficient Test of the U.S. Food and Drug Administration uses strain F.D.A. 209 as an indicator organism. Lethality occurs due to a 1:90 dilution of phenol in 10 minutes, but not in 5. Micrococcus pyogenes, var. aureus was used to establish the unit of penicillin.

Wethington and Fabian (1950) showed this organism to be resistant to pH changes. Scott (1955) found that the usual levels of acidity, nitrate, nitrite and salt in cured meat products would not prevent growth. Kinter and Mangel (1953) reaffirmed the resistance to low pH in salad dressings. Salmonella was much more sensitive to acid than Micrococci. Growth of Micrococci four hours after inoculation was found at pH 3.40. However, Levine and Fellers (1940) found pH 5.0 to be inhibiting, and pH 4.9 to be lethal, using acetic acid. Under any circumstance, this genus is more resistant to acid than most bacteria.

The resistance of Micrococcus to heat is greater than that of many of the nonspore-forming bacteria. Myhr and Olson (1956), Hammer and Trout (1928), Yesair, Bohrer and Cameron (1946), and Castellani (1953) described the thermoduric nature of micrococcal cells. This thermal resistance is much less by comparison with that of bacterial spores, but approaches the upper limits of pasteurization lethality.

Ingram (1955a)isolated a Micrococcus strain from canned ham that had been heated above 73°C for 60 minutes. This organism survived heating for 150 to 165 minutes and 165 to 180 minutes in separate tests at this temperature when suspended in bacon fat. When heated in Ringer Solution, however, and then in the fat, it survived for only 60 to 90 minutes. This report and the work by Yesair's group,

showed that dry cells in fat offer resistance to heating on the order of that required for dry sterilization. It is suggested that processing fatty foods may offer a problem. Ingram (1955b), in further work with ham, demonstrated the abundance of Micrococci in brines, resistance to curing agents, and increased resistance to heat in the presence of fat.

Lochhead and Jones (1938) showed Micrococci to be more resistant to freezing than other bacterial types occurring in frozen pack vegetables. They appear in greater numbers after incubation than in the fresh vegetable, indicating greater survival and the effect of a less competitive environment. Kiser and Beckwith (1942) showed that Micrococci could withstand freezing better than Achromobacter.

With radiation, the trend of somewhat greater resistance of Micrococci amongst vegetative bacteria continues. Proctor and Goldblith (1953) have determined the sterility doses for a number of these bacteria. Of eight organisms investigated, Micrococcus pyogenes, var. aureus was second to Streptococcus faecalis in resistance, and required 43.2 to 48.0 $rep_{93} \times 10^4$ to reduce an initial concentration of 12 to 18 $\times 10^7$ cells per gram to one cell per gram. As an extreme example of resistance, a Micrococcus strain has been found by Anderson, which will

survive two to three million rep. This organism has been isolated repeatedly from meat in one packing plant, and has been tentatively identified as Micrococcus rubens by Evans (1956).

5. Use as Indicator

The use of Micrococcus pyogenes, var. aureus is an important indicator of process effectiveness due to its relatively high resistance to a variety of processes. If a pasteurization operation or condition of holding will not permit the growth of this organism, then it is probably effective for the majority of other nonsporulating bacteria. This statement cannot be made into a universal concept, but can be applied to describe many situations. Certainly if Micrococcus pyogenes strains can be eliminated from a food material, then other vegetative organisms would undoubtedly be lessened in number to safe levels.

In addition to its relative resistance, Micrococcus pyogenes, var. aureus is the most common cause of food poisoning, as previously indicated. Thus, operations designed for its elimination would reduce this danger, as well as provide general safety from infecting microorganisms.

The use of this organism has value in another sense. Enterotoxin production depends upon the growth of the particular microorganism. As indicated, processing designed to destroy the organism may not destroy the enterotoxin. This danger makes the prohibition of any growth a necessity. Knowledge of this demands adequate handling care for any food before and after processing. Thus, this potential hazard could be a stimulus for the realization of the necessity for proper handling practices.

B. PROCESS OPERATIONS

1. Heating

Work involving the heating of commercial food products has been carried out along two channels: the high temperature methods, designed to sterilize canned packs as effectively as possible; and the low temperature, pasteurization methods for destroying most nonspore-forming organisms in heat labile foods.

Most of the quantitative work dealing with the lethality of bacteria has been based on the destruction of spores. Methods have been developed for the calculation of process time and temperature for canned foods. Many excellent discussions and descriptions of this work are available. Schmidt (1954) and Stumbo (1949) presented excellent reviews on this subject.

Work with low temperature treatment has not been so quantitative although the pasteurization process for milk has been exhaustively treated. The U. S. Public Health Service Milk Ordinance and Code (1953) provides standards, including bacterial populations, for different classes of milk and states that pasteurization consists of heating at 143°F for 30 minutes or for 15 seconds at

161°F. However, most of this information is directly applicable only to milk and, since data are lacking concerning the rates of lethality of commonly found organisms, cannot be extrapolated to give results for other food products.

Specifications of a processing scheme are dependent upon a number of factors. The first is the selection of an indicator organism. The conditions of milk pasteurization are dependent upon the destruction of Mycobacterium tuberculosis. (Tanner, 1944). The selection of Micrococcus pyogenes, var. aureus for the work involved in this thesis, as previously described, is ideal due to its generally high resistance relative to the class of nonsporulating bacteria.

The nature of the medium is important. For example, the thermal resistance of many types of microorganisms has been increased with high concentrations of soluble starch. This is shown by Wallace and Tanner (1931) for yeasts, Baumgartner (1938) for vegetative cells, and Anderson, Esselen and Fellers (1949) for bacterial spores. It has further been shown by Jensen (1944) with Streptococci and Yesair, Bohrer and Cameron (1946) with dried cocci that the presence of fat can sharply increase the resistance to heat, compared to heating in an aqueous environment.

The pH of the medium can have great influence on the tolerance of an organism to heat. Sognefest, et al (1948) found that spores of Cl. botulinim and Clisparogenes St. changed in their resistance to heat through a pH range of 4.5 to 9. Between pH 6 and pH 9 the changes were relatively slight, but below pH 5.5, resistance fell off sharply. Levine and Fellers (1940) and Skillinglaw and Levine (1943) reported studies of the effect of pH on the resistance of nonspore-forming bacteria.

Material within the medium may have a great influence on the resistance to heat. This varies with organisms and the medium. Salt may be used as an example of this. Yesair and Cameron (1942) found 3.5 per cent salt decreased the resistance of Cl. botulinum spores to temperatures below 230°F. Anderson, Esselen and Fellers (1949) showed that the heat resistance of B. thermoacidurans decreases with increases in the salt content of tomato juice. Sugiyama (1951) observed an increase in heat resistance with increased salt concentration. Esty and Meyer (1922) found a similar increase. Jensen (1954) covered the literature on this subject. He stated that, although the effects of salt vary, there seems to be general agreement that salt exerts a very protective effect against heat for the Micrococci species.

Alkaline salt solutions, however, have a great influence in lowering this resistance.

Of interest to this work is the effect of the curing ingredients. Yesair and Cameron (1942) found that the curing salts, sodium nitrate, nitrite, and chloride, all have inhibitory effects in different degrees both single and combined. It was found that there was a specific reduction in the heat resistance of Cl. botulinum in the presence of these curing agents. This was done by determining the lethality in cured and uncured meats. Jensen (1954) also reported on the lowering of heat resistance by the addition of curing ingredients. The effect described is more than just the bacteriostatis associated with the use of curing salts. It is actually the lowering of heating requirements to achieve the same lethality without the curing ingredients.

Williams (1929) did work with spores of B. subtilis on the matter of the influence of various materials on the resistance to heat. Media prepared from vegetable sources and isoelectric gelatin increased the resistance of the spores to heat. The addition of soluble carbohydrate, organic acids, or amino acids also increased the heat resistance.

The age of the cells to be destroyed also influences their resistance to heat. Age is taken to mean the various

stages of the growth phases, as well as storage age, measured in days. Sommer (1930) found the resistance of Cl. botulinum spores to be at a maximum at four to eight days. Curran (1934) studied aging up to one year, and found that the resistance increased with time. Gross and Vinton (1947) found a tendency for the resistance of a food poisoning strain of Micrococcus pyogenes, var. aureus grown for 14 days to be somewhat higher than that of cells grown for 48 hours. This work was carried out with meat substrates.

Within stages of the growth phases, Sherman and Albus (1923) and Sherman and Cameron (1934) found that vegetative cells show differences in their response to heating during various stages. Robertson (1928) and Stark and Stark (1929) found young cells to be more sensitive to heat than mature cells. Anderson and Maxwell (1936) showed that during the early logarithmic phase, streptococcal cells were more resistant. Ellicker and Frazier (1938) found the resistance of E. coli to vary. During the initial stationary phase, there was greater heat resistance, which decreased with reproduction, and was at a minimum during periods of rapid growth.

These varying factors point to the need for the experimental determination of lethality rates. Although indicative information can be found in the literature, direct application is not justified without experimentation.

There are many reports concerning the conditions of time, temperature, and environment necessary for the destruction of Micrococcus pyogenes, var. aureus. Some of the more pertinent of these are described below.

Topley and Wilson (1941) stated that Micrococci are among the more heat resistant nonsporulating bacteria. Many will withstand a temperature of 140°F for 30 minutes. They also describe the lactic and fecal Streptococci as having approximately the same heat resistance.

Castellani, et al (1953), working with food poisoning strains in turkey stuffing, showed that five strains did not survive 149°F (65°C) for five minutes. Two more heat resistant strains survived after being heated to 140°F (60°C) for 15 minutes, but did not survive being heated for 30 minutes.

Webster and Esselen (1956) did similar work with poultry stuffing as a medium. They showed Micrococcus pyogenes, var. aureus to have a z value (slope of the thermal death time curve) of 12.3°F, and a thermal death time of 15.4 minutes at 140°F. Similar studies were also carried out with two other organisms, Salmonella enteritidis and Streptococcus faecalis. The former had a lower resistance to heat, but one on the same order of magnitude. A z value of 10.1°F and a thermal death time of 19.25 minutes at 140°F was reported for this organism. Streptococcus faecalis was

considerably more resistant, having a z of 14.2°F, and requiring 67.2 minutes at 140°F for destruction.

Yesair, Cameron and Bohrer (1944) stated that 30 to 45 minutes at 131°F is sufficient to kill Micrococci. Stritar (1941), using the same strain, found that two minutes at 150°F was required to destroy one million cells at pH 7. Gross and Vinton (1947) studied the thermal death time of a highly heat resistant food poisoning strain. They found the z value to be approximately 9. The values for complete destruction were much higher than previously cited results. Meat substrates were used in this work.

Beamer and Tanner (1939) found survival at 13.8 minutes and lethality in 18.8 minutes at 150°F for a food poisoning strain of Micrococcus with a z value of 11.0. An initial concentration of 3.2 million cells was used in broth at pH 7.05. Jensen (1945) commented that "Staphylococci are killed at pasteurizing temperatures ... 61.7°C (143°F) ... for 30 minutes."

Myhr and Olson (1956) studied Micrococci in milk. Of 39 cultures isolated from laboratory pasteurized samples of raw milk, 13 survived 143°F for 30 minutes. They found z values to vary between 13.0 and 18.2.

Stritar, Dack and Jungewaelter (1936) developed a method for the pasteurization of custard-filled puffs and eclairs.

A temperature of 190.6°C (375°F) for 30 minutes eliminated any Micrococcus pyogenes, var. aureus present in the product without causing deteriorative changes. Lethal rates were not established.

Although there are many conflicting reports about the lethality of enterotoxic Micrococci, it is generally considered to be highly resistant to heat. Variation can be expected, as previously described and, as a result, the lethality rates, at a given temperature range, in a particular product, and under specific conditions of handling, must be experimentally determined.

2. Antibiotics

Pratt and Dufrenoy (1953) described the term antibiotic as the designation of:

> "... a metabolic product of one micro-organism that is detrimental or inimical to the life activities of other micro-organisms, usually even when present in extremely low concentrations."

It is the antibiotic's origin and great activity that have prompted its separation from purely chemical substances in this work.

There are four antibiotics of interest in connection with this work. Each is described as follows:

a. Aureomycin

Aureomycin is the trade name (Am. Cyanamid) for chlorotetracycline, which was first found by Duggar (1948). It is produced from Streptomyces aureofaciens, and has an antimicrobial spectrum of many Gram-positive and negative bacteria, rickettsias and several viruses.

Tarr, Southcott and Bisset (1950) used Aureomycin to prolong the storage life of fish. Continuing with this work, Tarr, Boyd and Bissett (1954) found it to be superior to fourteen other antibiotics when used to control spoilage of both fish and meat. Concurrently, work was being done with spoilage organisms, isolated from meat by Deatherage's group (1953), who showed the effectiveness of Aureomycin against these isolated organisms. Whole carcasses were processed, greatly extending the period of holding, including two days with no refrigeration. Kohler, Miller and Broquist (1955) showed the similar effectiveness of Aureomycin over other antibiotics in extending the storage life of poultry. Ziegler and Stadelman (1955) found similar results.

Smith (1952) and Blonde (1953) reported early work with vegetables. Smith reduced soft rot of spinach by applying the antibiotics, Streptomycin, Terramycin, and Aureomycin. Blonde found Aureomycin to be effective against several types of bacterial rot of potatoes.

From these beginnings, the feasibility of commercial use of antibiotics became apparent (Miller, 1956).

This antibiotic was the first to be allowed by the U.S. Food and Drug Administration for use with food materials. The Federal Register (Nov. 30, 1955) permitted its use with uncooked poultry in residual concentrations up to 7 parts per million (ppm). Usual cooking procedures will inactivate this concentration of the antibiotic so that the consumer will not be exposed to this material in the consumption of treated foods.

b. Terramycin

Terramycin, like Aureomycin, is an analog of the tetracyclines. It is the trade name (Chas. Pfizer) of oxytetracycline. It was found in 1949 by Finlay to be biosynthesized by Streptomyces rimosus. It has a broad spectrum of effectiveness, generally resembling that of Aureomycin. The early experimenters in the field of commercial food applications, described in Section a, also evaluated this material concurrently with the chlorine-substituted tetracycline. Essentially the two materials are quite similar. Approval for use in commercial processing of uncooked poultry was given by the U.S. Food and Drug Administration in the Federal Register on October 23, 1956.

c. Subtilin

Subtilin is a cyclic polypeptide produced by Bacillus subtilis having high antibiotic activity. Pratt and Dufrenoy described its discovery in 1944 and anti-tubercular effect in 1946-47, but cited Nicolle (1907) and Rappin (1912) as the first observers of the microbial effect in cultures. Humfeld and Feustel (1943) found antibiotic activity in crude extracts of B. subtilis (ATCC 6633) grown in asparagus medium. This activity was high against Gram-positive organisms.

Jensen and Hirschmann (1944), working with the same organism, reported the first complete work with subtilin, including development of synthetic medium, growth, extraction, tests for antibiotic activity, and chemical properties.

Salle and Jann (1945) studied the spectrum of organisms affected by subtilin. Although the previously cited work described the responding organisms, this was a more comprehensive evaluation. It was found that partially purified subtilin would inhibit growth of many Gram-positive bacteria, the Gram-negative Neisseria catarrhalis and N. gonorrhoeae, some acid fast bacteria, including Mycobacterium tuberculosis, and some pathogenic fungi.

Anderson and Michener (1950) described the successful processing of peas, asparagus, and corn using the combination of subtilin (5 to 10 ppm of 85 per cent subtilin) and mild heat. The asparagus was inoculated with Bacillus stearomthermophilis, a thermophile. Protection against bacterial spoilage was similar to that used in conventional steam heating. Burroughs and Wheaton (1951) showed the effectiveness of subtilin in preserving tomato juice inoculated with Bacillus thermoacidurans, another thermophile. Peas inoculated with Clostridium botulinum were found to have spoilage delayed in the presence of subtilin. In another aspect, Katznelson and Hood (1949) found that 0.01 to 1 ppm of subtilin inhibited Streptococci in cheese cultures.

The food industry, however, is not as interested in subtilin as it is in other antibiotics. Tarr, in the work cited for Aureomycin, found that 50 ppm of subtilin were valueless in the preservation of fish during experiments which showed the usefulness of the tetracylines. Denny, Bohrer and Cameron (1951) compared packs processed by subtilin and mild heat with packs prepared by regular steam processes. The physical quality of the former packs was superior. Packs inoculated with Clostridium botulinum and other putrefactive anaerobes were quite resistant to subtilin treatment, and, as a group, the mesophilic bacteria

tended to be resistant. Thermophiles, however, particularly the flat sour types, were found to be very sensitive. Cameron and Bohrer (1951) concluded that subtilin does not meet an important criterion of acceptability by the food industry, that of laboratory and pilot-scale demonstration of destruction of spores of Cl. botulinum and other spoilage organisms. This statement is from the point of view of the National Canners Association, which is concerned with the sterilization of canned food products. There may well be less general applications in which subtilin could be used to advantage.

The addition of subtilin is not permitted in food materials at the present time.

d. Nisin

The discovery of this antibiotic is very interesting. Like the other antibiotics, nisin was found by observing an inhibition of microorganisms. But, unlike so many others, nisin was found to be a common ingredient of a common food, cheese. It is almost as if an important food application had been developed simultaneously with its discovery.

The first observation was by Rogers (1928), who noted the inhibition of Lactobacillus bulgaricus

by certain strains of Streptococcus lactis in the preparation of Yogurt. Whitehead (1933) made a similar observation, and began studies to isolate and characterize this material. Mattick and Hirsch (1944 and 1947), Wheater, Hirsch and Mattick (1951 and 1952), and Hirsch and Grinsted(1951 and 1954) developed and described the production, extraction, isolation, and study of antibiotic and elementary chemical properties, and were responsible for its name, nisin.

Nisin is a metabolite of certain Streptococci and the product of its growth. The natural environment of these organisms is cheese, where they are used as starting cultures. Nisin is a large polypeptide with a molecular weight of ca 10,000.

Unlike the tetracylines, nisin is not a broad spectrum antibiotic. It is inhibitory to species of the genera: Staphylococcus, Streptococcus, Neisseria, Bacillus, Clostridium and Corynebacterium.

Nisin is used commercially in England, France and Holland on a large scale in the manufacture of cheese. (Hawley, 1955). The chief concern in cheese making is a defect known as "blown cheese", resulting from the growth of clostridial species. Control by lowering the pH and increasing the salt content has not been found to be satisfactory. (Hood and Smith, 1951). Pasteurization of pro-

cessed cheese tends to lower the nonsporulating organisms, but not the spores. The redox potential of cheese is usually too low to allow the germination of aerobic spores. In one million pounds of processed cheese treated with nisin, no blowing was seen although control batches indicated that about 10 per cent would have blown under normal conditions had nisin not been added.

The addition of nisin is not permitted in this country because it does not meet the criteria for the approved antibiotics, that of consistent destruction in foods before consumption. The basis for use in other countries is that ptyalin and trypsin will destroy nisin (Hawley, 1955), and that in its narrow spectrum and instability at the acidity of the intestines, it would have no effect on the resident flora. This is reported by Barber, Braude and Hirsch (1952) for pigs, and Coates, et al (1951) for poultry. Toxicity tests by Frazer and Hickman (1956) showed no deleterious effects when nisin was fed to rats for three months at concentrations of 20,000 and 40,000 times expected human dietary levels. A further argument is that nisin is produced in cheese by organisms that also inhabit the intestinal tract, thus the human has probably been exposed to nisin under normal conditions.

Interest in this work with these four antibiotics is concerned primarily with the destruction of Micrococcus pyogenes, var. aureus in meat media, with treatment by

the combination of antibiotics with heating. This is because food uses currently require the inactivation of the antibiotic before consumption. The tetracyclines are destroyed in heating, which is the basis for their approval for use. Subtilin has been shown to be resistant to heat, but this resistance is a function of pH, the rate being greater at pH 6.4 to 7.1. Lewis, et al, (1947) showed the condition for these losses to be incompatible with normal culinary practices. The same holds true for nisin. (Hawley, 1955).

Because these two materials are protein in nature, it is possible that there may well be means to inactivate them. For example, protein may become denatured under some conditions of frozen storage; and perhaps a combination of pH control, heating conditions, and frozen storage would produce inactivation.

Concerning its effect on Micrococcus pyogenes, var. aureus, nisin is cited as having a very great lethality. Godkin and Cathcart (1952) reported that 10 to 20 ppm of subtilin, 0.4 to 1 ppm of Terramycin, and 0.6 to 1 ppm of Aureomycin would retard the growth of food poisoning strains of Micrococci for 24 hours in inoculated, autoclaved custard fillings. Subtilin, 50 to 100 ppm, was the only material that would retard both the normal heat resistant flora and the inoculated Micrococcus in nonautoclaved fillings.

3. Chemicals

In this work, chemicals are defined as materials added to a substrate, which under conditions of normal use, are not found in that substrate. Thus, by this definition, nitrite is not a foreign chemical to ham, but would be to roast beef. Antibiotics could properly belong in this group, but have been placed in a separate category.

a. Sodium Nitrite

The role of sodium nitrite in meat curing is based on three phases: it acts to produce the typical cured meat color by a series of reactions culminating in the formation of nitric oxide myoglobin, which upon cooking, becomes nitric oxide myochromogen; it is a factor in the production of the cured meat flavor; and it has some bacteriostatic effect. It is permitted as an additive to meat in concentrations not to exceed 200 ppm. In addition to the nitrite itself, muscle tissue and halophilic bacteria convert some of the added sodium nitrate to nitrite. Jensen (1954) discussed cured meats in considerable detail.

Work has shown that sodium nitrite, within the permissible concentration, is not too effective against bacteria. Bittenbender, et al (1940) found that Micro-

coccus pyogenes, var. aureus was not killed by exposure to 38.8 per cent sodium nitrite in 10 minutes in a pH range between three and eight.

However, Tarr (1941 a,b) has shown that under certain conditions, sodium nitrite may be effective. Below pH 7, bacteria are susceptible to its action, depending upon the species type. He found that the growth of Micrococcus species was inhibited by 200 ppm of nitrite at pH 5.8. It was also found that the growth of Clostridium botulinum and Cl. Sporogenes was similarly arrested.

Loss of nitrite in meat may occur. Brooks, et al (1940) have shown that nitrite may be lost in cooking above 212°F. This loss depends on the initial concentration. For 50 per cent destruction, 13 minutes were required for an initial concentration of 30 ppm, but 120 minutes for 589 ppm.

Zobell (1932) has shown that some microorganisms have the ability to destroy nitrite. This seems to be a step in the path of reduction reactions from nitrate to ammonia.

b. Hydroxylamine

Sodium nitrite is related to hydroxylamine. Lindsey and Hines (1932) showed that various bacteria can produce hydroxylamine from nitrates and nitrites. Woods (1938) found similar evidence.

Although the reputation of this material for possible food applications is not widely known, its use in foods has appeared plausible for some time. Budde (1908) obtained a U.S. patent for the sterilization of milk by adding 0.07 per cent hydroxylamine, and freeing it by the further addition of hydrogen peroxide.

Jensen (1954) showed that hydroxylamine is very inhibitive to many bacteria associated with meat, including Micrococcus, Achromobacter, Pseudomonas, Serratia, and Bacillus. With nutrient agar as the substrate, inhibition was caused by the presence of 1:20,000 (50 ppm of Hydroxylamine). Much of this effect was lost, however, with the addition of 0.1 per cent glucose.

Tarr (1945) found hydroxylamine to be excellent for the control of fish spoilage. A concentration of 0.001 per cent (10 ppm) provided inhibition for two weeks in nutrient broth at 25°C of ten species isolated from fish. Eight of these cultures were inhibited for longer than two weeks by 25 and 50 ppm. A food poisoning strain of Micrococcus pyogenes, var. aureus was inhibited beyond two weeks in the presence of 25 ppm of hydroxylamine. The organism was suspended in nutrient broth, and was incubated at 37°C and pH 5.9. At pH 7.1 and pH 7.5, 50 ppm were required for inhibition, and this inhibition was only transitory. Clostridium botulinum was inhibited above

pH 6.3 by 50 ppm, but at pH 5.9, only 25 ppm were required. The effect of pH was not constant for all species, nor within reasonable limits, was it a strong factor.

Moncrief (1946) described hydroxylamine as odorless, but Jensen (1954) stated that an undesirable flavor may be imparted to high fat foods by its presence.

c. Ascorbic Acid

There are several valuable effects associated with the addition of ascorbic acid to both vegetable and flesh foods.

Kelley and Watts (1957) studied the effect of ascorbic acid as a reducing agent on cured meats. They found that this material catalyzes the production of nitric oxide myoglobin. In addition, it regenerates this red pigment on the surfaces of cured meat in the presence of residual nitrite. It further has a protective effect on the surface against oxidative changes. Hardy, Blair and Krueger (1957) found that the browning occurring on specific surfaces of canned luncheon meats was due to oxidation by nitrite, catalyzed by iron ions. Contact with the iron was possible at the edges of the can and at seams. Ascorbic acid eliminated this defect.

In addition to the protection of color, ascorbic acid acts as a synergist in the prevention of fat rancidity. This was shown by Tarr (1947), Bauernfeind, et al (1948), and DuBois (1949). Most of this work was carried out with fish. Not only was rancidity delayed, but the yellowing of fillets was prevented as well. Bauernfeind, Smith, and Siemers (1951) developed a method for spraying or dipping fish, with ascorbic acid solutions, using concentrations of 0.5 to 2.0 per cent. A dip solution of 0.25 to 1 per cent thickened with a colloidal material was suggested for use.

Bauernfeind, Smith and Parman (1954) have written an excellent review article on the use of ascorbic acid in meats. The authors stated that ascorbic acid is not a preservative for spoilage due to bacteria or fungi. This article does show, however, the advantages of using this material in meat foods.

d. Acetic Acid

The selection of acetic acid in preference to other organic acids for control of spoilage is the result of two factors. Acetic acid as vinegar is inexpensive to obtain, and its use is common as a preservative in food products.

In addition, Levine and Fellers (1940a) showed that the bacterial action of acetic acid is more than that attributed to pH alone. In another study (1940b), they found that acetic acid was inhibitory to Micrococcus pyogenes, var. aureus at pH 5.0, with an inhibiting acidity of 0.03 per cent. It was lethal at pH 4.9, with an acidity of 0.4 per cent. They also found that these cells were killed within 15 minutes in 5 per cent brine and 0.17 per cent acetic acid.

Erickson and Fabian (1942) found that acetic acid had both a greater preserving action and bactericidal effect than either citric or lactic acids at the same pH levels.

Nunheimer and Fabian (1940) evaluated acids in terms of sensitivity of Micrococcus pyogenes, var. aureus. They found that of five organic acids and hydrochloric acid, acetic acid had the greatest preserving and bactericidal effects, and that hydrochloric acid had the least.

Gomutputra and Fabian (1953) found that 4 per cent distilled vinegar destroys both Micrococcus and Streptococcus on meat surfaces with no appreciable flavor of vinegar detectable after cooking.

The resistance of Micrococcus pyogenes, var. aureus to the effect of acid was previously discussed in Section A-4.

e. Sorbic Acid

The use of sorbic acid as a mold inhibitor was patented in 1945 by Gooding. It is in general use as a fungistatic agent in foods.

Melnick, Luckman, and Gooding (1954) proposed a likely mechanism of action. It is believed that the unsaturated fatty acid chain of sorbic acid inhibits the enzymatic dehydrogenization of saturated fatty acids.

Sorbic acid has high selectivity for the catalase positive molds, and the catalase negative Lactobacillus and Leuconostoc are unaffected. Thus the use of sorbic acid as a mold inhibitor in cheese is ideal because there is no interference with the cheese-making process.

Emard and Vaughn (1952) developed a differential medium containing sorbic acid, having a high selectivity for the catalase negative organisms. In this work they studied the sensitivity of many bacteria to sorbic acid. Three strains of Micrococcus pyogenes, var. aureus in liver broth and agar were slightly affected by 0.10 per cent sorbic acid, and did not grow at all within five days in media containing 0.12 per cent sorbic acid. They also studied the effect of pH combined with 0.07 per cent sorbic acid in glucose broth. The inhibiting pH without sorbic acid for the three strains was 4.3. With 0.07 per cent sorbic acid,

the inhibiting pH was 5.9. Clostridium botulinum, catalase negative, was uninhibited by sorbic acid.

f. Benzoic Acid

Benzoic acid and its more water soluble sodium salt is widely used as a preservative to prevent yeast spoilage in foods. Dunn (1947) reviewed many applications of benzoates which usually are used in a concentration of 0.1 per cent.

Sodium benzoate has its greatest effect at a pH range of 2.5 to 4.0, which was shown by Cruess and Richert (1929). Rahn and Cohn (1944) found that benzoic acid was about a hundred-fold more effective in acid solution than at neutrality. Fellers and Harvey (1940) found that benzoic acid and its derivatives could be effectively used to preserve fish at pH 4 to 4.5.

g. Propionic Acid

Propionic acid is a pungent compound used in a salt form with sodium or calcium ("Mycoban"). In this form, it has a cheese-like flavor, which is not objectionable. Propionates are often used in bakery and dairy products as a fungistatic agent.

Olson and Macy (1945) used propionates to impregnate parchment wrappers for butter and cheese. Jacobs

(1951) described the use of propionates for the inhibition of mold and rope in bread.

h. Essential Oils

The use of spice material for the preservation of foods has been of interest for many thousands of years. Blum and Fabian (1943) studied 32 spice oils and seven components of yeasts, and found that oil of mustard (allylisothiocyanate) was consistently superior as a preservative. Kosker, Esselen, and Fellers (1951) found that 10 ppm of allyisothiocyanate, both in buffer solution and apple and grape juices, had a marked effect upon the heat resistance of mold and yeast forms, but a lesser effect upon the resistance of Bacillus thermoacidurans. They also found that this material had a greater preserving action than had several other spice oils. Anderson, Esselen, and Handleman (1953) found great variation in 12 essential oils, with mustard oil being one of the more effective. The inhibitory power of these oils was increased at lower pH ranges, pH 4 to 5 being much more effective than neutrality.

i. Quinones

The possible food use of quinones of the

Vitamin K series to prevent spoilage began with some observations in dental science. Armstrong and Knutson (1943) found that acid production in saliva was arrested when quinones were present. They tested further and found that quinones effectively reduced initial low counts of Micrococcus pyogenes, var. aureus in saliva. Armstrong, Spink, and Kahnke (1943) found that 2 methyl-1-naphol-4-amino HCl was oxidized to 1,4 napthoquinone. This compound was tested against Micrococcus pyogenes, var. aureus with an initial count of 3500 organisms per gram. It was found that 0.61 mg per 100 cc inhibited growth for 24 hours, 0.91 mg per 100 cc inhibited growth for over 48 hours.

Atkins and Ward (1945) found that the analogs of Vitamin K should be in a form sufficiently unstable to hydrolyze readily to release free quinone. Dam (1948) wrote an excellent review article on Vitamin K, defining its chemistry clearly.

For food uses, Pratt, Dufrenoy, and Pickering (1949) found that Vitamin K_5, in concentrations of 0.001 to 0.03 per cent, was effective in preventing the growth of yeasts, fungi, and bacteria. They described the first use of this compound as a preservative for fruit and seeds. Ribereau-Gayon and Peynaud (1952) found that Vitamin K_5 delays the growth in wine of yeast and acetic acid bacteria. Sulfur dioxide treatment concurrently complements the Vitamin K effect by preventing its oxidation.

4. Ionizing Radiations

The processing of food by the use of ionizing radiations is similar to heat processing in that both are dependent upon energy input. However, whereas heating creates a condition of thermal energy transfer, radiation is characterized by the energy of high velocity particles or rays. Because heat is not generated with radiation, the term "cold sterilization" is used.

Proctor and Goldblith (1951a) evaluated the available forms of radiation, and considered that only gamma-radiation and electron radiation are applicable to food problems. The source of gamma-rays is an isotope; that of electrons, a high energy electron accelerator. Gamma-radiation, as quanta of energy, has great penetration into matter because it has no mass to either be absorbed by physical means or be attracted by electro-magnetic phenomena within the irradiated material due to the fact that it has no magnetic charge. High energy electrons, because of their mass and negative charge, have very limiting depths of penetration, dependent upon their energy levels. The uses for isotope sources are those processes that require great penetration; with food materials isotope sources would be used for products having great mass, such as ham or material with a volume of a normal sized can. Electrons are useful when only

surface effects are desired, or when the material is, or can be compounded into, a shape with one thin dimension on the order of 0.5 inch. For food irradiation, Bellamy and Lawton (1954) described the use of electron radiation. Crean, et al (1953) and Duffey (1953) discussed the use of isotopic sources.

As with heating, either pasteurization or sterilization by radiation may be sought in the treatment of foods. The limiting factor again is the stability of the quality of the product to the deteriorative influence of radiation. When undesirable physical or chemical changes occur at sterilization doses, the lower pasteurization dosages may be satisfactory. The product is usually then refrigerated.

The pasteurization of meat slices by high energy electron beams is a process of promise. Morgan (1957) described the good use the Army has shown with ham that had been irradiated at sterilization levels (greater than 2 million rep). Beef is less stable at these high levels, and has fair acceptability.

Schweigert, Doty, and Niven (1955) showed that the difference in flora of cured and fresh meat indicates that cured meat products require higher doses. The flora of cured meat predominates in Lactobacilli, Micrococci, and yeasts, some of which require a lethal dose of 90,000 rep.

The pasteurization of beef can be successfully conducted at doses of 50 to 100,000 rep.

Pasteurization is possible because the dose ranges required to destroy Micrococcus pyogenes, var. aureus are considerably below the levels that cause severe physical changes. Proctor and Goldblith (1953) showed that, whereas this organism has a relatively high resistance to radiation, 43.2 to 48.0 x 10^4 rep were sufficient to reduce 12 to 18 x 10^7 organisms per gram to a level of one organism per gram. Schweigert, Doty, and Niven (1955) indicated that 11,000 rep is required to destroy this organism, and that 150,000 rep is required for Clostridium botulinum. Fram, Proctor, and Dunn (1950) found Micrococcus pyogenes, var. aureus to be relatively radio-resistant, with a MLD (37% survival) of 3600 to 4400 rep with x-rays.

At higher dose levels, even possibly with pasteurization doses, organoleptic changes may be brought about in foods. Such defects as off flavor, color changes, and textural effects may be encountered. It is possible to minimize some of these undesirable effects if the changes are not too severe. This may be accomplished by the addition of chemicals, such as ascorbic acid, to minimize oxidative effects. Proctor, Goldblith, et al (1952) evaluated such biochemical materials. The addition of

seasoning materials may also aid in masking off flavors produced in foods by irradiation treatment.

Proctor and Goldblith (1951a,b) have written review articles comprehensively defining the radiation process, critically describing sources, and showing various applications and problems with the emanating radiations.

5. Freezing

There are two important concepts involved with the freezing of foods. The first is an extension of storage life by slowing down physical and chemical changes. The second is concerned with microbiology.

There is agreement that the freezing process is not a sterilization action, and that after thawing, the microbiological population will institute the same spoilage actions, which would occur in the unfrozen product. There is substantial evidence, however, to show that microorganisms respond with variable lethality and biochemical alterations to freezing. It is with this thought, in addition to an extension of storage life from the standpoint of other types of changes that this work is of interest.

Magoon (1932) described the lethality of bacteria, molds, and yeasts to freezing. He felt that although

large numbers of the microbial population are destroyed by freezing, a small proportion can always be found which is unaffected. He also discussed the psychrophilic forms which can even grow at freezing temperatures. Berry and Magoon (1934) demonstrated the growth of Pseudomonas fluorescens at -4°C. Smart (1934) found similar results with eight species at 16°F.

The freezing temperatures are important in affecting microbial lethality. Winter and Wilkin (1947) found that the bacteria in egg products held at 0°F or above were destroyed to a much greater extent than when held at much lower temperatures. Schneiter, Bartram and Lepper (1934) found similar evidence with eggs. Sulzbacher (1950) showed a general picture of low temperature lethality with frozen pork. He demonstrated a greater coliform destruction at 25°F (-4°C) than at 0°F (-18°C). These results are interesting in that they indicate that lethality is not a direct function of the freezing temperature. Jensen (1954) cited the freezing point of meat, defined as the point at which ice crystals are formed, as 29.5°F. The degree of reduction which can be expected in freezing varies greatly. Berry (1933) froze berries in brine, and noted the degrees of destruction. He found a destruction after four months of storage of 40 per cent at -20°C, 89 per cent at -10°C, and greater than 99 per cent destruction at -2°C. This again shows noncorrelative temperature effects. Prescott,

Bates, and Highlands (1932) observed similar variation in the degrees of lethality.

Kiser and Beckwith (1942) were concerned with the flora of frozen fish. They observed a 59 per cent reduction in total flora at -20°C after 15 days. They commented on the high resistance of Micrococci to freezing. Jones and Lochhead (1939) froze corn, isolated 50 strains of Micrococcus pyogenes, var. aureus, and demonstrated the survival of this species after freezing.

In another paper, Lochhead and Jones (1938) explained large percentages of Micrococci that occur in frozen pack vegetables after thawing on a basis of the resistance of this genus to freezing. The freezing reduced substantial populations of the other flora, and thus the Micrococci, with little physical damage remained viable in greater relative numbers, and grew unhampered. They also showed that the ability to produce enterotoxin after freezing may be affected. Among 18 enterotoxic strains, which were frozen, only eight produced enterotoxin after thawing at 68°F. No enterotoxin was produced at temperatures from 40 to 50°F.

Another point of interest is the time required to initiate growth after freezing. Hucker and David (1957) studied 113 strains, including 38 Micrococci and 30 Gram-negative rods, which they found constituted the flora of

chicken pies. The time required for growth after freezing was determined to be eight hours at 90°F, 12 hours at 70°F, 25 hours at 45°F, and 70 hours at 35°F.

Straka and James (1935) carried out work with the inoculated peas which were subsequently frozen. They found that there is some lethality of Clostridium botulinum spores during freezing. On thawing and incubation, the only samples of 100 heavily inoculated packs showing evidence of Botulinum toxin were six, held for two days at 80°F, and three at 50°F for one week. They concluded that there is no danger of botulism from frozen foods that are properly handled. Diehl (1945) emphasized that correct procedures during food operations virtually ensures safety from any botulism hazard.

The greatest effect with low temperatures is the sensitivity of young cells to cold shock. Sherman and Albus (1923) noted that E. coli cells in early growth stages had a high lethality when exposed to temperatures of 0 to 37°F. Sherman and Cameron (1934) used E. coli, grown for 90 to 180 minutes at 45°C. When transferred to media at 10°C, 95 per cent of the population was reduced within one hour. This effect is apparently concerned with the early reproduction stages, as it is seen in early logarithmic growth.

III.

PACKAGING CONSIDERATIONS

III. PACKAGING CONSIDERATIONS

A. PACKAGE REQUIREMENTS

1. General

The purpose of a package for the samples under consideration in this work is two-fold. The package should permit isolation of the environment of the product during processing, and following processing, continue this isolation to prevent the entrance of microorganisms. The package should protect the product from physical and chemical changes such as might be caused by air and desiccation. Successful processing is dependent upon proper packaging. Package design has been simplified of late because of the wide range of materials and combinations of materials available commercially.

The selection of a packaging method depends upon:

a. The nature of the product

b. The effects of the process on the product

c. The requirements of handling and storage.

Selection is carried out by listing the package requirements of these three factors for the process under study and by finding the materials and methods which fulfill these needs.

The steps used in package selection for the various processes are described in this section.

2. Bread Slices

The work of Pence, et al (1955) and unpublished work carried out by the author show that there are no special packaging problems associated with the freezing of bread. Storage lives of months are found with loaves held at 0°F and lower. The loaves investigated were table white bread with expanded cell structure, and were packed in waxed paper. This type of white bread is the most susceptible to the staling processes because of the fine texture and open structure. For several years, a coarser white bread has been successfully frozen in a commercial operation (Arnold, 1954).

The freezing of individual or paired slices, however, depends more critically upon successful packaging because there is no protection of the crumb by the crust, as there is in a whole loaf. With slices, the entire staling complex and oxidative factors are accelerated, and effective packaging is the only method of prevention.

The requirements for packaging and freezing bread slices are:

a. Low moisture vapor permeability to prevent crumb desiccation, and the resulting staling effects of texture change and starch recrystallization.

b. Low permeability to gases for the maintenance of a vacuum or positive inert gas pressure, whichever is indicated in the process. This is primarily carried out to minimize the presence and prohibit the admission of oxygen, which is responsible for oxidative flavor changes and may be associated with staling.

c. Heat sealing ability for ease of construction and to ensure either a vacuum or positive pressure.

d. Grease proofness to prevent package staining and possible accelerated rancidity because of greater exposure to light or air. Bread crumb has sufficient free shortening to cause such a problem.

e. Physical properties that resist changes at freezing temperatures during long periods of frozen storage.

f. High mechanical strength against tearing, rupture, etc. Without sufficient strength, a protective package would be needed, causing unwieldy bulk and prohibitive costs.

3. Meat Slices

The packaging of meat slices offers somewhat more of a problem than does the packaging of bread. In addition to being frozen, the packaged unit must be exposed to a prior processing operation, which generally will tend to lower the quality of the product. The package must aid in minimizing this.

The requirements of packaging for meat slices are:

a. Resistance to changes induced by the processing operation, i.e., radiation or heat.

b. Low moisture vapor permeability to prevent dehydration in frozen storage or the condensation on the slice due to exposure to atmospheres of higher temperature and humidity.

c. Low permeability to gases for the maintenance of a vacuum or positive inert gas pressure, if either are indicated for the process.

d. Heat sealing ability for construction and ensurance of either a vacuum or positive pressure if needed.

e. Physical properties resisting change at freezing temperatures, during long periods of frozen storage.

f. High grease proofness to avoid staining or accelerated rancidity by the meat slices which have a high free oil content.

g. High mechanical strength to prevent tearing, rupture, etc., during handling.

B. EXPERIMENTAL: PACKAGE DESIGN AND TESTING

1. Bread

For the packaging of bread slices, the degree of vacuum and temperatures required for long-term frozen storage was first evaluated. This was carried out by packaging individual slices in enameled quarter-pound sardine cans. Fresh slices of commercial sandwich-type white bread were trimmed to fit the can, resulting in a headspace of three-eighths of an inch. Duplicate slices were coated on one side with butter in order to observe any effects that butter might have, as butter is the commonly used spread for sandwiches.

The cans were sealed with and without vacuum in a mechanical sealer with a vacuum chamber. The pressure in the chamber was approximately 27 inches (vacuum). The samples were held at 68°, 38°, 0°, and -40°F for one month.

Before evaluation, all samples were held at room temperature (ca 75°F) for several hours to allow the frozen samples to thaw. Each can was opened just before examination. Organoleptic and physical characteristics were used as indices of quality.

The results showed that none of the samples could be described as being of high acceptability, and that only the slices held at -40°F were of fair acceptability. The difference between vacuum and air packed samples at this temperature was slight, and consisted only of a weakly bread-like odor of the vacuum-packed slices. The air-packed slices, although similar physically, had a disagreeable odor. The texture of the bread held at -40°F was slightly tough, but was acceptable.

At none of the other three conditions was the bread even questionably acceptable. The texture was somewhat dry and very tough, and the odor did not resemble that of bread but showed not only a loss in character, but also very high off odors. Vacuum-packed samples held under these conditions showed no observable difference.

It was interesting to note that the slices spread with butter were generally in better condition than the unbuttered samples. After storage at 0°F and -40°F, the buttered slices were acceptable, whereas as noted, the plain slices were not acceptable at 0°F. As the buttered face was on top, the slices were less exposed, but this protection did not seem to be great enough to produce a marked difference in quality. The spread, itself, apparently was unchanged.

It was considered that the problem lay in the re-- moval of as much air as possible from both the ambient environment and the crumb cells.

To accomplish this, a flexible package was made from polyethylene-laminated Cryovac (Dewey & Almy). This package was made in an envelope shape to just enclose the bread slice. A vent 1.5 inches long led a channel to the outside; the entire package was heat sealed. Through this vent, air was withdrawn by a tube that was connected by flexible vacuum tubing to a water jet vacuum source. The intention was to exhaust the air within the package, pinch off the vent, and heat seal the vent close to the package, thus holding the atmosphere.

It was found that upon subjecting bread slices to a strong vacuum, the crumb structure collapsed. The cells of the crumb rapidly lost their rigidity. To stop at a point before collapse would be unsatisfactory because an insufficient vacuum would have been produced.

The problem was met by packaging the slices in open envelopes, as before, but the slices were frozen before a vacuum was applied. The freezing gave rigidity to the crumb structure, which supported the cells during the vacuum application and sealing. This method was successful and individual slices became frozen quite rapidly (within 30 minutes at 0°F). The vacuum must be applied within a few minutes before the slices begin to thaw if carried out at room temperature. In practice, slices were frozen at 0°F for 30 to 40 minutes, withdrawn from the freezer, subjected to vacuum treatment, sealed in the package, and returned to the freezer for storage.

The initial freezing had no deleterious effects upon the quality of the slices. Also, on thawing, the crumb did not collapse, suggesting that either the gas pressures within the crumb and package had become equalized or that the structure only collapses when the gases are rapidly withdrawn.

The selection of the laminate was based on information from the Modern Packaging Encyclopedia (1956), listing the properties of all commercial films. Because the laminate is of polyethylene, the package is heat sealable. For holding bread slices, the best possible material, in terms of gas and water vapor transmission, was needed. Polyethylene, itself, would not have been satisfactory because it has a high permeability for gases; Cryovac has a very low permeability. Satisfactory results were also obtained with a polyethylene laminate of Mylar (DuPont).

A test similar to the one described was done with slices being vacuum-treated by this freezing method. The high vacuum samples, in all cases, were superior to those held in air or low vacuum. The only defect found in the samples held above 0°F was a lessening of the bread odor. Samples held at 68°F showed no obvious off odor. In this test, like the others, the product was held for one month at 68°F, 38°F, 0°F, and -40°F.

This packaging method is also applicable to positive

pressure packing, using, for example, nitrogen to replace the air in the crumb cells. This technique depends on the creation of a partial vacuum, followed by flushing the package with nitrogen gas, removing this with high vacuum, and finally, filling the package with nitrogen. This method was not used because the vacuum procedure seemed suitable. It was considered only to show its feasibility. The package will maintain a positive pressure for at least one month.

It is considered that the packaging of a sandwich by this method for frozen storage is quite feasible. For holding periods of several days prior to consumption, the slices do not require frozen storage. The first stages of bread staling are marked by a loss of moisture by the crumb, followed by a change in the physical nature of the starch. Final effects, such as oxidative rancidity and flavor changes do not occur in the early phases. Because this package does not allow moisture to escape from the crumb, and the amount of air present is limited, informal testing has shown that slices so packaged may be expected to retain acceptable quality for periods up to a week at room temperatures if they do not become moldy. Flavor changes limit this kind of storage. For periods of a day or two, the frozen-vacuum process is not needed. The pro-

tection of the package will allow the slice to retain its physical character. After two days, oxidative effects begin to gradually reduce the organoleptic value.

2. Meat

The packaging of meat slices is greatly influenced by the methods used to process the slices. Although there is some similarity between heating and radiation treatments, each process is treated separately.

a. Air Oven Heating

For heating in an air oven, the effects of temperatures up to 300°F were investigated. Heating causes some separation of fat and water from the slices, which indicates the need for a highly grease-proof package. A fat stained package has an unsightly appearance, and oxidative rancidity can occur more rapidly. Resistance to change with temperature must also be found in the packaging material.

The material used for packaging bread is not satisfactory for high temperature treatment. The maximum use temperature for polyethylene is 180°F; for Mylar, 490°F; and 290°F for Cryovac.

It was decided to use heavy duty aluminum foil of 0.0015 inches to hold meats in frozen storage. This material has a maximum use temperature of 700°F, and is highly greaseproof. Vacuum was not considered because it was believed that should slices be used with bread as sandwiches, the first step would be to process the filling separately in its package, and then to combine it with the bread. This could be done by including the filling within the vacuum-processed bread package or by enclosing both within a master package, which could have a vacuum if such were needed. Preliminary storage tests showed that tight foil packaging for frozen meats would suffice for several months of storage at 0°F.

The package was made in the shape of an envelope. The foil was cut, folded in half, the slice inserted, and one side and two end flaps were made by folding the top and bottom edges twice. The folds were made so that the folded edges came to the edge of the meat slice, but did not overlap it. Thus all of the heat transfer areas had but a single thickness of foil between the slice and the outside. This eliminated the hazard of having areas under the fold which might have a heating lag due to the necessity to transfer heat through several thicknesses of foil, increasing the heating required for bacterial destruction in these areas.

An advantage of this package is the ease of opening. The folded flaps are easily torn off by tearing the flapped edge from one corner of the folded edge, and continuing along the folds. The entire flap, on three sides, is removed in this tearing action. The slice is simply removed by lifting the top fold.

It was found that this package met the requirements for heating. Samples were heated within the range of temperature and time requirements for the processing operation, after which they were frozen. The only defect was fat and moisture within the inner surface, but this was not too objectionable. Unheated and unfrozen samples, held for several hours at room temperatures, showed similar evidence of fat and moisture.

b. Ionizing Radiation

For irradiation with electrons, which have very limited penetration, a package must be as thin as possible, and the material from which it is made should have a low density. The absorption of cathode ray energy is a function of both density and thickness. Any increases in thickness or density of materials would increase the absorption of energy, permitting fewer electrons to reach the product and reducing the penetration of the electrons passing through the package. For this reason, a film is preferred to al-

uminum, which is superior to tin plate.

A major consideration in the selection of a packaging material for irradiation processes is the stability of that material to radiation. The ionizing radiations used at the levels for food sterilization, one to three million volts (M.e.v.), can induce changes in the polymer structure of plastic films. Reynolds (1957) discussed packaging for radiation. He reported that the chlorinated polymers tend to degrade with radiation. This would eliminate the use of Cryovac (vinylidene-chloride).

The material selected for packaging was polyethylene-laminated Mylar, (0.0005" Mylar - 0.002" Polyethylene). Nagel and Wilkins (1957) described the properties of Mylar. They stated that irradiation at sterilizing doses has no effect on its mechanical and physical properties. Mylar also has a minimum use temperature of -80°F, making it suitable for freezing.

The amount of energy absorbed by this material during irradiation is negligible. Manganese glass dosimeters were irradiated beneath the surface of a single thickness. Any changes in the glass could not be measured. The procedure is described in Section V.

Packages were made into envelopes to contain one slice of meat. The dimensions varied, depending on the dimensions of the meat. The slices had a mean thickness of 2.1 mm. This is described in Section IVc. Packages were first

made approximately 6 x 4 inches, and were heat sealed on three sides. The slices to be irradiated were placed in this envelope, and the package was sealed into a form-fitting shape, reducing the amount of excess internal space.

There were no observable changes during the radiation treatment and subsequent freezing. The package was opened by cutting off the edges. A tear flap for easy opening was not designed although it might be relatively easy to do so. The package was found to be quite satisfactory.

This package could also be modified to hold a vacuum or positive gas pressure if either were required. Groninger, Tappel, and Knapp (1956) have indicated that a vacuum or positive inert gas pressure may be desirable during the irradiation of meat.

IVa.

PRELININARY CONSIDERATION OF THERMAL PROCESSING

IVb.

DETERMINATION OF THERMAL DEATH TIME OF MICROCOCCUS PYOGENES, VAR. AUREUS SUSPENDED IN BOILED HAM SOLIDS

IVc.

THE DESTRUCTION OF AN ENTEROTOXIN PRODUCING STRAIN OF MICROCOCCUS PYOGENES, VAR. AUREUS AND RESIDENT FLORA IN BOILED HAM AND ROAST BEEF SLICES BY AIR OVEN HEATING

IVd.

THE RELATIVE HEAT RESISTANCE OF THE TWO STRAINS OF MICROCOCCUS PYOGENES, VAR. AUREUS USED IN THIS WORK

IVa. PRELIMINARY CONSIDERATIONS OF THERMAL PROCESSING

A. INTRODUCTION

The preservation of foods by heat is one of the most commonly used processes throughout the food industry. Considerable understanding has been developed of the quantitative aspects of the destruction of microorganisms and enzymes by heat. This information has been gainfully applied to the solution of many important processing problems.

One of the canning problems that has never been solved involves the relative inability of heat processing to properly preserve large masses of meat; ham is a typical example. Ham presents a large mass of low thermal conductivity having a variety of mirororganisms distributed within its structure. To reach satisfactory lethality levels in the center of a ham with heat would, in most cases, require sufficient overheating of the external portions to result in a totally unacceptable product. Many canned items are packed in brine to facilitate heating by both conduction and convection.

The broadest concept of this work evaluates the answer to the following question. If ham is eventually to be used in slices, and cannot be successfully heat treated, why should it not be possible to slice the ham prior to processing and then heat process the individual slices in order to render them relatively bacteria free?

The processing of slices to eliminate Micrococcus pyogenes, var. aureus is dependent upon the response of that organism to heat. Extrapolation from the results given in the literature is difficult for several reasons: reported results differ, substrates and methods vary, and there is a lack of lethality rate data which may be used to establish thermal death relationships. It is for this reason that the thermal death rate data in this work were obtained at temperatures as near as possible to those used in the experiments of processing.

The application of this data lay in the actual processing of slices under simulated commercial conditions. The investigation was carried out for both roast beef and boiled ham.

B. METHODS OF HEATING

Preliminary work was conducted to evaluate two methods of heating meat slices, and their suitability for application to operating conditions. Both conventional heating and dielectric heating methods were studied.

1. Conventional Process

For this work, dry heat was used, the product being heated in an air oven with a hearth. Heating was supplied by resistance coils at the top of the oven and beneath the hearth. The product was placed on the hearth during the heating. Under ordinary conditions, dry heating has less lethal effect than has moist heat. However, the product has moisture, since it is within a closed package; the vehicle of heating, therefore, is actually a moist atmosphere.

Samples of sliced, cooked roast beef and ham were packaged in aluminum envelopes and processed for varying periods of time at different temperatures. The meat slices used were of a standard thickness of 2.1 mm (average). This thickness of meat is satisfactory for sandwich use. It is also quite suitable for the work with radiation. It was considered to be important to

standardize the thickness for both heat and radiation processing, so that relative merit of the two processes could be determined without the introduction of variables which would be caused by meat slice thicknesses.

It was found that in a tightly folded package the physical characteristics of sliced roast beef and sliced ham did not alter appreciably under the following conditions:

185°F ..... 25 minutes
200°F ..... 15 "
250°F ..... 10 "
300°F ..... 8 "

These conditions allow for heating sufficiently to achieve a high degree of bacterial lethality, as shown in Section IVc.

An attempt was made to combine both the processing and the cooking operations of the roast beef. Raw beef slices were packaged in aluminum foil envelopes, and heated at different temperatures until cooked to the same extent as roasted slices.

As a further refinement, some of these raw slices were soaked in four per cent vinegar for one minute before being cooked to complement the lethal effect of the heating. The slices were drained well before being packaged.

In both cases, the products were unsatisfactory. The meat was cooked by a boiling action. Much moisture

was rendered from the structure of the meat, which became tough and stringy. The taste of meat heated in this manner was characterized as that of stewed meat.

The vinegar dipped slices had a distinct taste of vinegar. Under conditions of cooking in which the meat is not tightly packaged, it is quite likely that the acid might be volatilized. If this were done, however, the slices would be completely dried by the cooking process.

Because of these unsatisfactory results, the combination of cooking and processing was not pursued further.

However, the processing of packaged roasted beef slices, as well as boiled ham slices, by dry heat was entirely satisfactory. This work was developed further, and is described in Section IVc.

2. Dielectric Heating

As another source of heating, the effects of dielectric heating were studied. The Raytheon "Rada-Range" was used at medium power (approximately 800 watts) for varying periods of time, from 30 to 180 seconds. The advantage of this apparatus is that it provides the possibility of quickly heating a food throughout the entire thickness. Heating quickly reaches a maximum of 212°F, and will not exceed this unless the environment

is enclosed in a tight package which would allow for the build up of high internal pressures.

For this preliminary work, the products were packaged in polyethylene. Slices of boiled ham and raw and roasted beef were processed in an envelope-shaped package.

It was first evident that the desired results would not be attained by heating the product in a sealed package in this case. The heating action was quite rigorous, and the water vapor evolved and the gases that expanded during heating caused the package to balloon until it burst.

To overcome this, one end of the package was left unsealed during heating, with about a four-inch elongation on the unsealed side. This was considered to be sufficient to prevent the entrance of micro-organisms after processing and before sealing. Packages so processed were not satisfactory. Fat and water rendered from the meat flowed or condensed on the un-sealed flap, which sealed it in some places, and where present on the flap after heating made heat sealing im-possible because of the liquid interface.

The next step was the use of the same package together with an aluminum cylinder positioned in such a manner as to form a channel through the unsealed flap.

In theory, this would reduce the amount of fat and water deposited on the inside of the package. The cylinder could be removed after heating in the Rada-Range, and the package sealed. Condensate and fat would be trapped, in this case, on the inside surface of the cylinder, not on the surface of the package. The results obtained by this method were more satisfactory; the package could be more effectively heat sealed after processing. Although aluminum does not absorb the energy from the magnetron because of its low dielectric constant, it did become heated by conduction from the hot moisture and fat. Occasionally, the package became sealed to the cylinder, making its removal almost impossible without ripping the film.

Under no circumstances, with Rada-Range heating were the raw slices successfully cooked and processed simultaneously. The quality of meat slices heated in this manner was characterized by the same defects, and much poorer than the slices similarly processed by oven heat, which were themselves unacceptable.

The quality of roast beef and boiled ham slices was much lower than meat slices heat treated in hot air. Apparently, the rigorous action of this heating method was too severe for these thin slices. In all cases, the air oven produced better processed slices having much less water and fat rendered.

For these reasons, heating by dielectric energy was discontinued, emphasis being placed on conventional methods of heat treatment.

IVb. DETERMINATION OF THERMAL DEATH TIME OF MICROCOCCUS PYOGENES, VAR. AUREUS SUSPENDED IN BOILED HAM SOLIDS

A. EXPERIMENTAL PROCEDURE

1. Equipment for Determination of Thermal Death Time

The heat treatment equipment used was that designed by Stern (1953) which is fully described in his doctoral thesis. In brief, the equipment consisted of two stainless steel pans with a device that would transfer a sample holder from a position over the pan containing the heating medium to a position over the pan containing the cooling medium in 0.4 second. The samples were placed in tubes which were inserted in small holes in the sample holder, and were held in position by the bent ends of the tubes. The tubes holding bacterial suspensions to be treated were thus fully immersed in each bath in turn. The hot bath contained mineral oil as the heating medium while the cold bath contained water for cooling. Experiments by Stern (1953) and Farkas (1955) have determined that the heating lag in tubes such as those used in this thesis was only

six seconds before a point of 0.1°C below bath temperature was reached.

The sample tubes used were the same as those described by Stern except for the inovation of the "glass bridge", devised by Farkas (1955), and employed by Kan (1956). For this work, further modifications were made in the reported procedures.

Soda glass melting point tubes (1.5 - 2.0 x 100 mm), used as sample containers, were soaked in sulfuric acid-sodium dichromate cleaning solution overnight, then rinsed with tap water and distilled water, and finally dried in a hot air oven. The ends of the tubes were bent at right angles in the gas flame of a microburner in order to place the tubes in the tube carrier of Stern's equipment.

Further preparation of the tubes varied from that of the methods cited. The tubes were placed bent-top upright in cotton-plugged Eerlenmeyer flasks, and were sterilized in a steam autoclave at 15 psig for 15 minutes. They were then dried in a hot air oven before being used.

2. Preparation of Ham Homogenate

Boiled ham was obtained in bulk. No trimming or selection of particular portions of the ham was carried out except for the elimination of connective tissues. The

ham was sliced, cut into pieces, weighed, diluted 2:1 with distilled water, and blended in a Waring Blendor at high speed for two 10-minute periods, the samples being cooled for 15 minutes between the two blending periods. Sufficient ham was used to provide 200 ml of homogenate. The homogenate was distributed in 50 ml aliquots into 200 ml Erlenmeyer flasks, which were then plugged with cotton and over-wrapped with aluminum foil. The flasks were then sterilized by being heated in free-flowing steam for two hours, and were frozen until used.

It was shown that two hours of heating in free-flowing steam was sufficient to destroy all viable micro-organisms present. The plate counts on T.G.E. agar obtained after processing were as indicated in the following:

0 hour	10^5	organisms/gram
1 hour	10^1	" "
2 hours	0	" "

Homogenates were prepared weekly and frozen. Prior to use, the frozen homogenate was thawed in free-flowing steam for 30 minutes. The flask was agitated by hand to break up clumps of ham solids and cooled before inoculation.

3. Preparation of Bacterial Suspension

The organism used was Micrococcus pyogenes, var. aureus F.D.A. 209, ATCC No. 6538. It is widely used as a standard indicator organism.

The organism was grown for 24 hours in 150 ml of Nutrient Broth at 37°C. Cultures were started weekly from agar slants, and daily by the transfer of 1 ml of 24-hour culture to the broth.

The inoculum was prepared by centrifuging 50 ml of broth culture for 5 minutes at 12,500 rpm in a sterile stainless steel centrifuge tube. The supernatent was discarded, and the packed cells were re-suspended by the addition of 50 ml of ham homogenate to the tube, followed by rigorous shaking. The suspension was blended at high speed in a Waring Blendor for one minute to ensure proper cell distribution and smooth texture. Ten ml of the suspension were transferred to a sterile 20 ml shallow dish, covered with aluminum foil. The capillary tubes were filled from this dish.

4. Sample Preparation

The capillary tubes were filled by creating a vacuum within each tube while the straight end of the tube was beneath the surface of the homogenate. Vacuum was applied by withdrawing the plunger of a 1 ml tuberculin syringe fitted with a 24-gauge, 1½-inch needle. The needle was flamed before use. The outside diameter of this needle was slightly less than the inside diameter of the tube, providing a sufficient seal for the vacuum. Where the

clearance was too great to make a good seal, the needle tip was moistened slightly in sterile distilled water before inserting to make a water seal. This water was unable to merge with the homogenate within the tube to dilute it.When there was insufficient clearance, the tube was discarded. Vacuum was applied at the tip of the short end of the tube.

The tubes were sealed after the desired amount of homogenate had been drawn up. Sealing was carried out with an air-gas flame by heating a small area close to the end, and withdrawing the end with forceps quickly to form a seal. Tubes were held horizontally, allowing the homogenate to move along the tube away from the end being sealed. Tubes were held in this position until the glass cooled. This prevented any bacterial reduction due to contact with hot glass.

To make the bridge, the centers of the tubes were heated in an air-gas flame until the glass was fused, then the tubes were withdrawn from the flame and slightly stretched in order to obtain a straight smooth "glass bridge". This bridge was necessary to prevent the rising of the bacterial suspension due to the expansion of the heated air in the top of the tube.

Measurement of the amount of homogenate was carried out on a weight basis, which required weighing before and

after filling on a balance. Weighings were made to the nearest milligram. As the filled tubes were weighed after sealing, it was necessary to include the weight of the ends removed in the sealing operation.

This procedure was considerably more cumbersome than volumetric methods, that allow direct filling of volumes of about 0.03 ml by the use of a 1 ml graduated syringe. However, it was considered that the weighing method had two great advantages:

(1) A much higher solids content could be used in the suspended medium. Whereas Kan (1956) was only able to use a 3.5 per cent medium, this method employed solids on the order of 12 to 15 per cent. It was felt that the highest possible solids content should be obtained for an accurate view of substrate effects.

(2) The weighing method was more accurate than the volumetric because of the difficulty to correctly dispense 0.03 ml aliquots of a relatively thick liquid.

After filling, the tubes were heated in the Stern apparatus.

5. Counting of Treated Samples

After heat treatment, the tubes were held in a chilled water bath to prevent growth until plating. Before counting, each tube was wiped with a cellulose wipette to remove most of the adhering oil and water from the hot and cold baths. Each tube was then rinsed and soaked in the chemical cleaning solution for at least one minute, rinsed in tap water and sterile distilled water, and finally broken off at, or below, the bridge into a test tube containing 5 ml or 10 ml of sterile distilled water, or to a dilution bottle containing 99 ml, depending on the lowest dilution required. Each tube was crushed in the dilution water with a sterile glass rod. Proper dilutions were made, and were then plated.

In some cases, up to four tubes were combined into a single sample for counting. This has been indicated in the Tables of Results and was done to determine, as far as was possible, the high lethalities achieved with increasing time of heating at a given temperature.

Counts were made in accordance with Standard Methods for the Examination of Dairy Products (1953) using Tryptons Glucose Extract Agar. Cultures were incubated at 37°C.

6. Treatment of Data

Duplicate counts were averaged for each tube. In each run, four tubes generally were used for each experimental point. The counts were calculated and expressed on a 1-gram basis. Because the mean counts represented survival after heating, the mean count of four unheated control tubes was considered to represent 100 per cent survival for each run. In calculating survival, this mean count was used as the divisor. The calculated "per cent survival" values were plotted semi-logarithmically at corresponding heating times. Regression analysis was used in the determination of the "best straight line" (Appendix $\overline{X}$ - F), through 100% survival.

For the determination of a thermal death time curve, the times required to reduce bacterial populations by 90 per cent at each heating temperature (D value) were plotted on semi-logarithmic graph paper according to the corresponding temperature. The curve was drawn through the resultant points.

B. RESULTS AND DISCUSSION

Survival curves for each temperature are shown in Figures 1 through 6. These curves are combined in a composite graph, Figure 7. The D value was found from each curve. D represents the time required for the destruction of 90 per cent of the bacterial population at the stated temperature. These values are listed in the following table.

Heating Temperature (°F)	D Values (minutes)
125	36.25
140	1.74
155	0.73
170	0.51
185	0.25
200	0.10

Based on these heating times, a type of thermal death time curve was constructed by plotting the logarithm of the D time in minutes with the corresponding heating temperature. This curve is shown in Figure 8.

The nature of this curve is very unusual. The curve is described by two segments, the first a straight line from 125°F, changing in slope after 140°F, and thereafter continuing as a curve to 200°F.

This curve is not a straight line throughout the heating range. Such semi-logarithmic plots are usually shown as a straight line.

By analysis of the straight line portion, it is possible to determine the slope, z value, by defining it as the temperature required to traverse one log cycle of time. The z value thus obtained is 11.3. This agrees with the values cited in the literature.

The second portion of the curve, however, is not in agreement with results found in the literature. An explanation can be postulated to account for some of this variation. It is very difficult to find accurate heating methods,with a means of quickly heating and cooling at a definite temperature for very brief periods, which can be used for thermal death time work. It is much more convenient to work within a temperature range, the lower temperatures of the range requiring about 30 minutes for complete destruction, the upper temperatures five or ten minutes. These are approximate figures, but for Micrococci, a non-spore forming genus, temperatures of about 125° to 145°F are usually used, for determination of thermal death time. A measured come-up time (thermal lag) can be found, and may then be used in correcting by allowance for lethality during this period. If it is assumed that a method has a come-up time of three minutes, a correction of 58 per cent

may be made for lethality, calculated to a 1.74 minute corrected zero time. This may be subject to some experimental error, but the resulting error may only be of the order of a few tenths of a minute, which relative to a 10 or 20 minute heating period, would only be an error of a few per cent. This would be well within the limit of the experimental error.

For this reason, it is likely that work has been done in this type of temperature range. Also, it approximates the usual pasteurization conditions (143°F for 15 minutes) used for milk, and is therefore of interest.

For the purposes of this work, however, information was desired concerning the behavior of these cells, when present in the foods which were being investigated, at comparatively high ambient temperatures because high temperatures were used to process meat slices. It was also considered important to evaluate the organism at the lower ranges because it was only at these temperatures that comparisons could be made with lethality values found in the literature. The capillary tube method permits these measurements to be made through a broad temperature range.

The usual practice in constructing typical thermal death time curves is to determine survival at several convenient temperatures, and then either plot D values or F values corresponding to the temperatures used to find the

slope of the thermal death time curve, as was done in this work.

The curve obtained by using D values has been referred to as the "phantom" thermal death time curve because it has direction, but no position. (Ball, 1943). Olson (1950) and Schmidt (1954), however, have suggested the name of thermal resistance curve, since they consider that it represents the real measure of thermal resistance because it has both direction and position. Ball's (1928) original thermal death time curve used thermal death times which are taken as the time required to reduce to concentration of viable cells at 250°F over a range of eleven log cycles. Drawing a curve based on D values theoretically eliminates the factor of the concentration of organisms, whereas that based on F values depends on initial numbers. (Bigelow and Esty, 1920). D values were used in this work because its purpose was not sterility, but the establishment of rates of lethality for pasteurization, implying the presence of surviving organisms.

When thermal death time curves are drawn from D values, the lines are drawn through the plotted points, and extrapolated to both lower and higher temperatures, where actual measurement would be too time consuming (lower temperatures) or too short for accurate determination (higher temperatures).

The results of this work show that in the case of Micrococcus pyogenes, var. aureus this extrapolation may be found in error because the straight line relationship was found to hold only at the lower temperatures. Rahn (1945) showed that the z value varied with temperature, lending substantiation to this claim.

The data of Webster and Esselen (1956) have been used for comparative purposes because the D and z values are in close agreement within the range of 125° - 140°F. This work was carried out with poultry stuffing as a medium. It was based on a plate count method for survival, instead of the use of the classical method of heating a number of tubes, and recording the survival in terms of numbers of tubes showing growth or no growth. It is considered that the plate count method is more accurate although considerably more tedious because it deals with numbers of bacteria.

Figure 9 shows the thermal death time curve derived from the data of these authors. For comparative purposes, the curve found in this work is also indicated.

A theoretical discussion of a possible explanation for this change at high temperatures is included in the next section. The answer would seem to lie in the realm of the mechanics of the heating process. Extrapolation of the straight line segment of this curve results in

a heating time of 0.00003 minutes at 200°F are required to destroy 90 per cent of Micrococcus pyogenes, var. aureus in ham homogenate. This time is so short that it might not permit heat to be transferred into the cell, and cause whatever processes are required for bacterial death, or loss of reproduction. Although temperatures of the order of 200°F have very great lethal effects upon vegetative bacterial cells, it is unlikely that times as short as 0.00003 minutes (1.8 milliseconds) would be sufficient to destroy 90 per cent of the organisms.

This work has provided information concerning the lethality of Micrococcus pyogenes, var. aureus. The response to heat at high processing temperatures, as well as the characteristics of the organism at lower temperatures are considered.

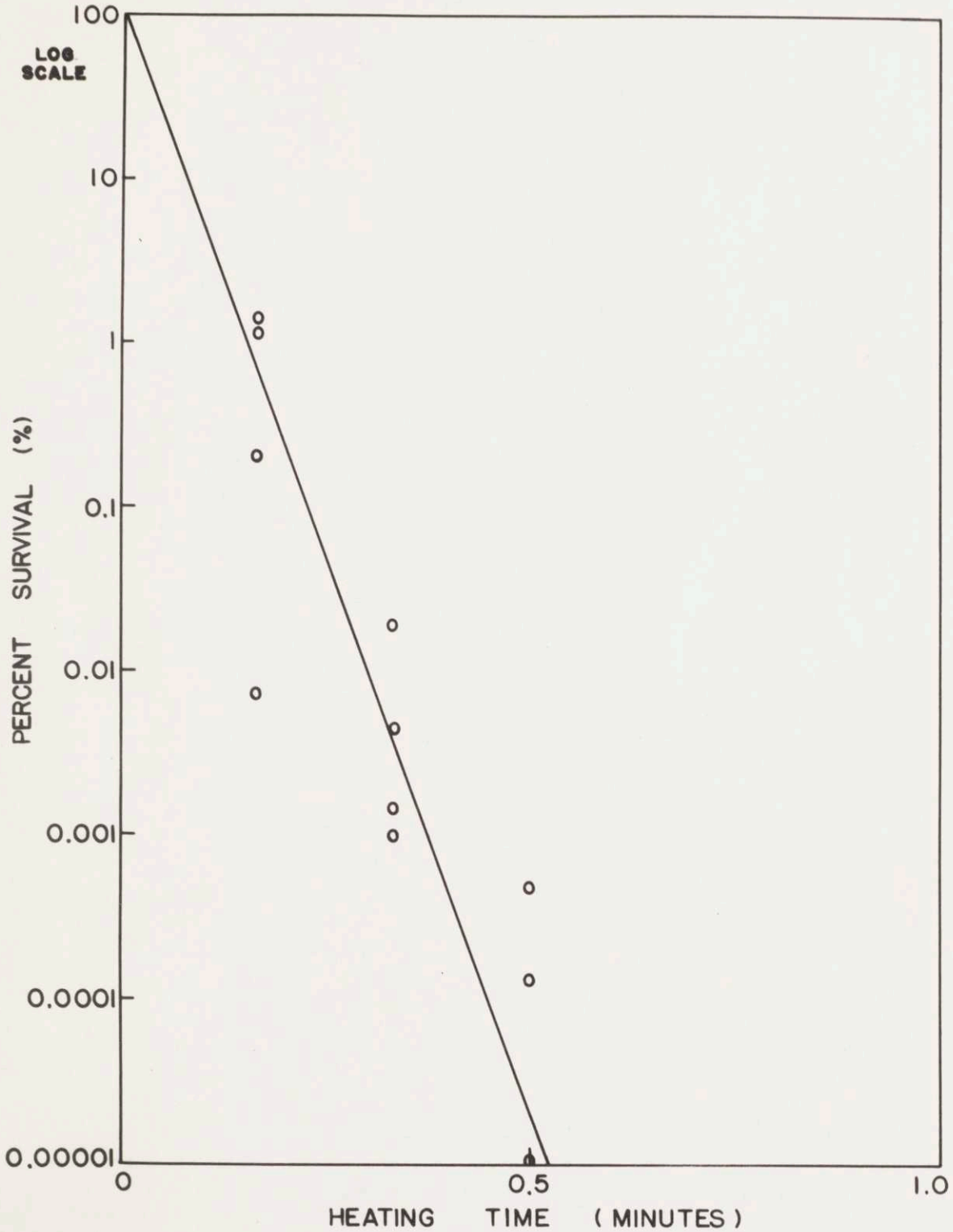

Figure 1. Survival Curve of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated at 200° F in Capillary Tubes.

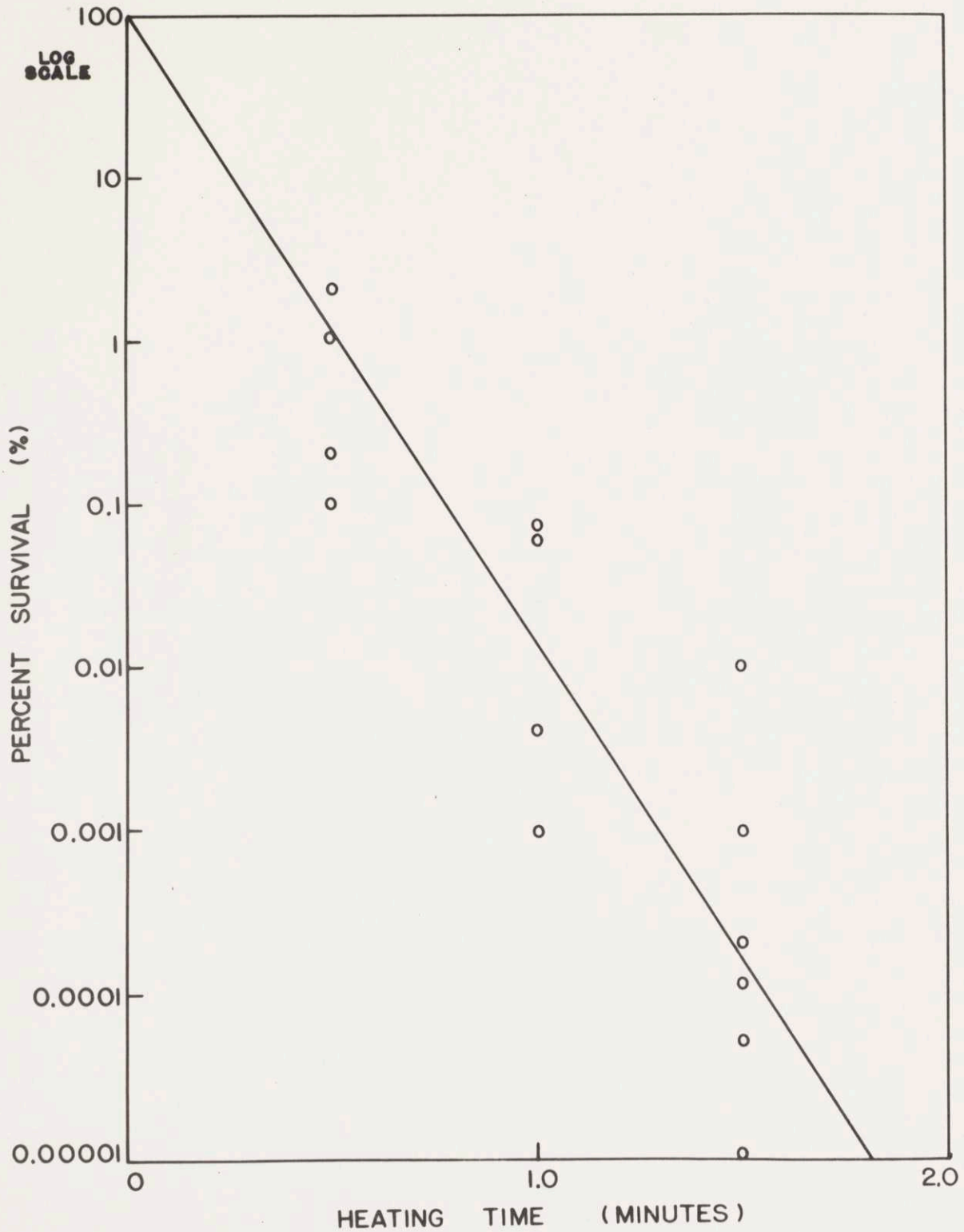

Figure 2. Survival Curve of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated at 185° F in Capillary Tubes.

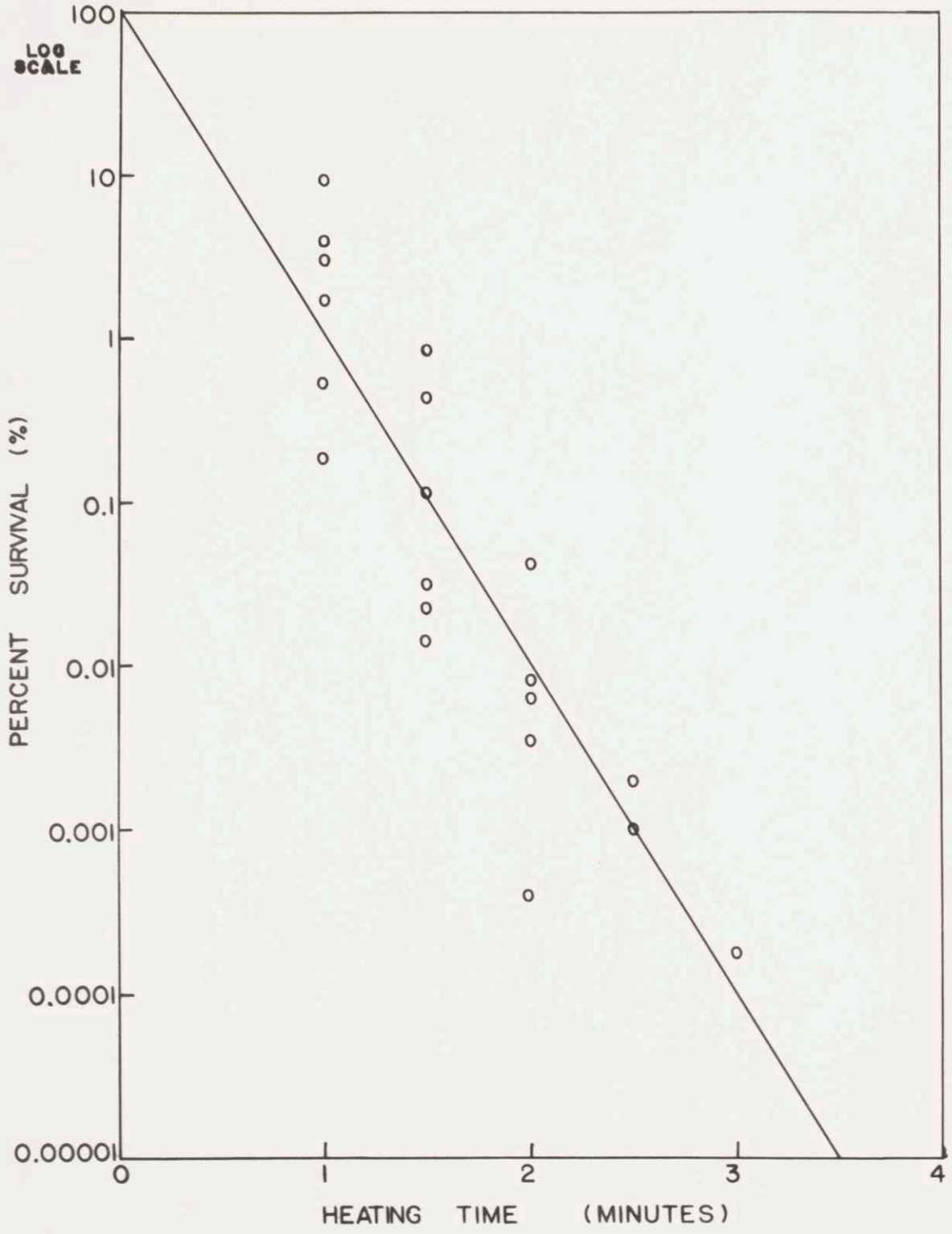

Figure 3. Survival Curve of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated at 170° F in Capillary Tubes.

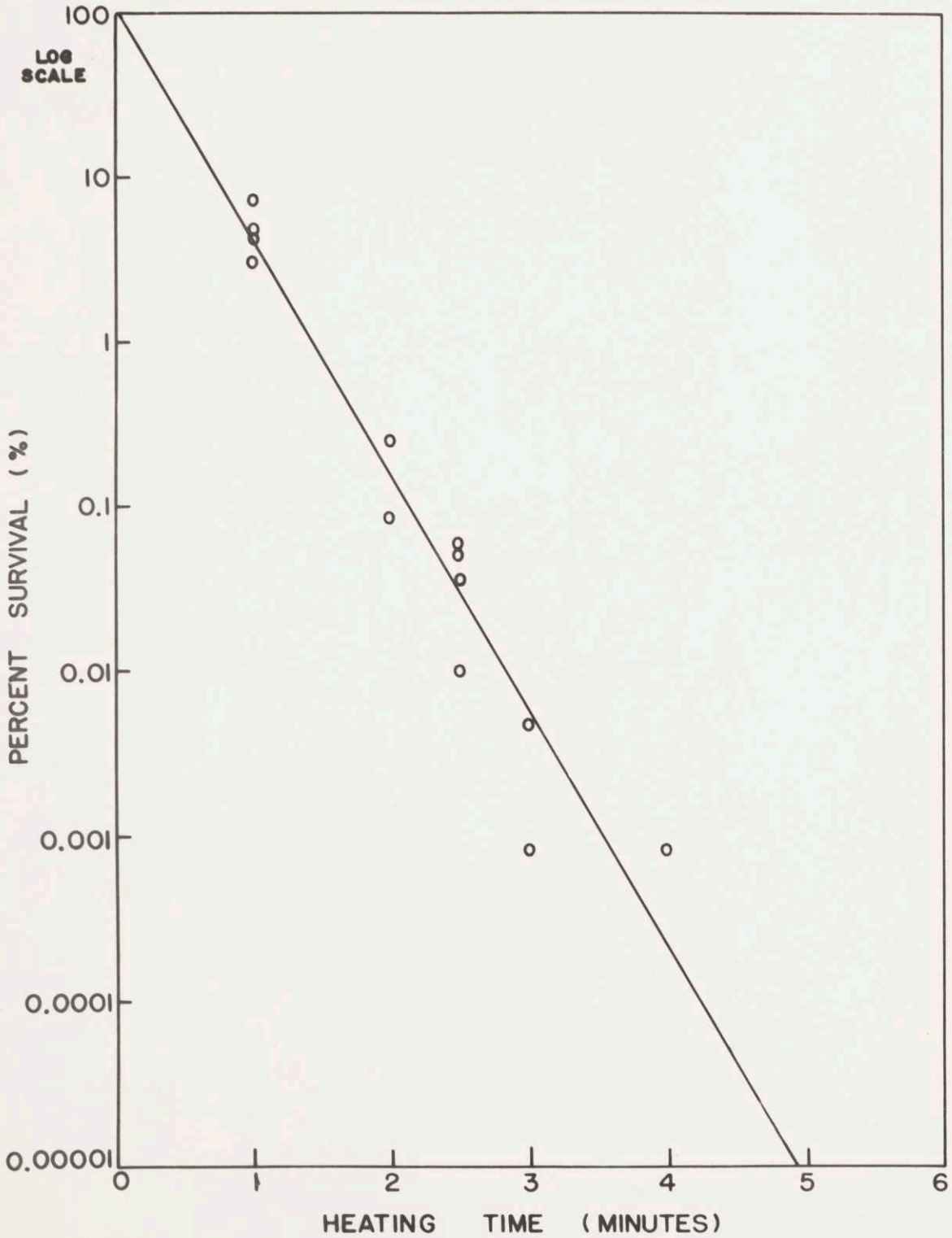

Figure 4. Survival Curve of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated at 155° F in Capillary Tubes.

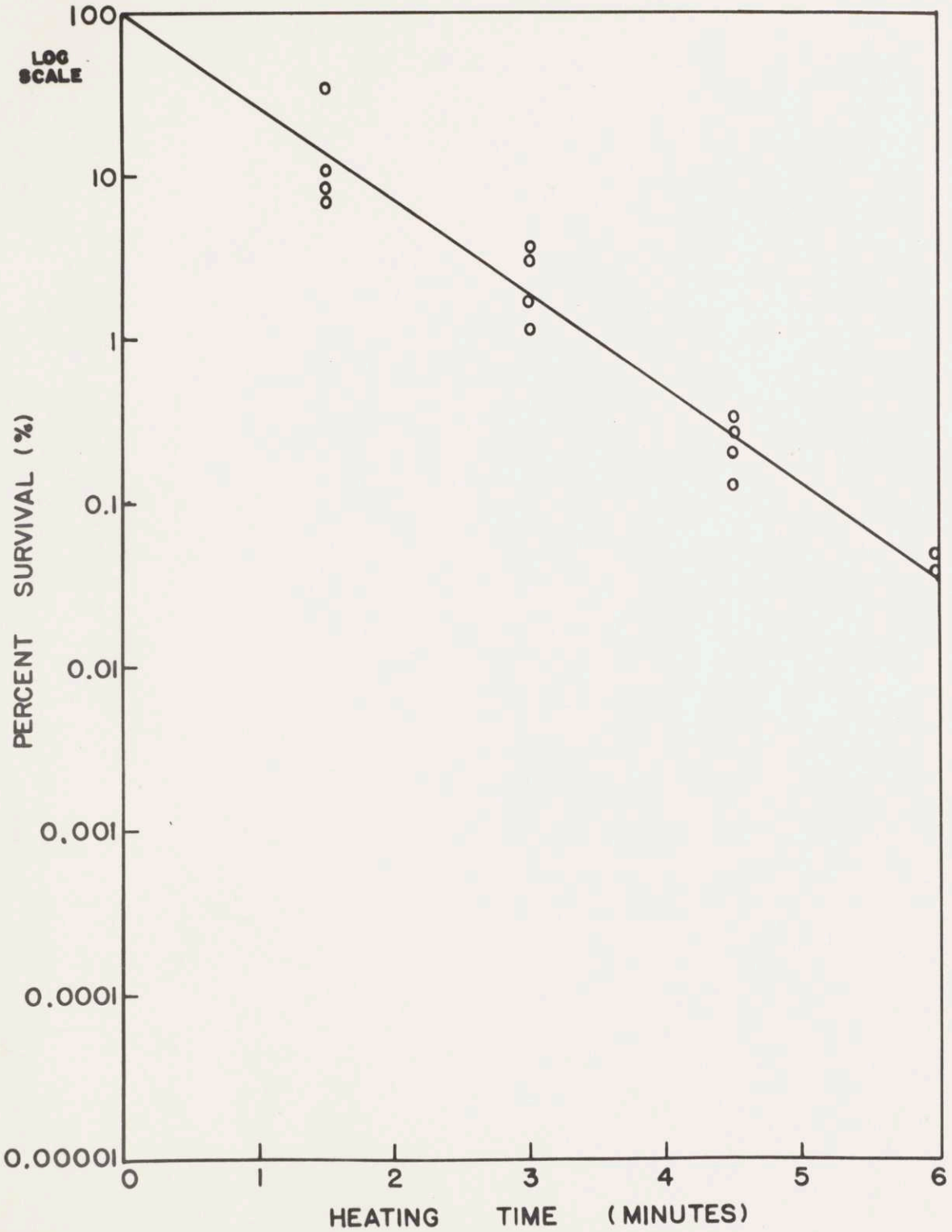

Figure 5. Survival Curve of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated at 140° F in Capillary Tubes.

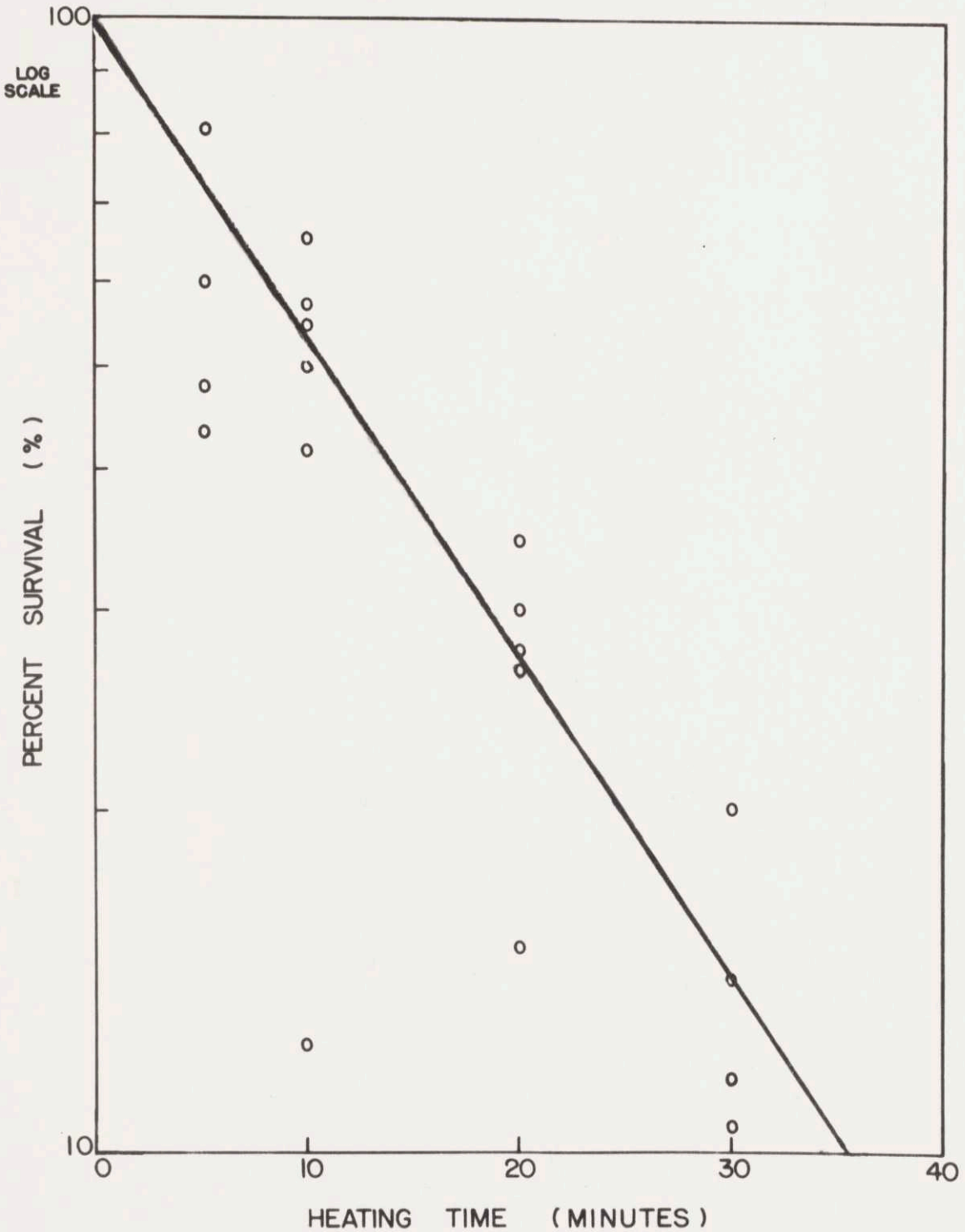

Figure 6. Survival Curve of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated at 125° F in Capillary Tubes.

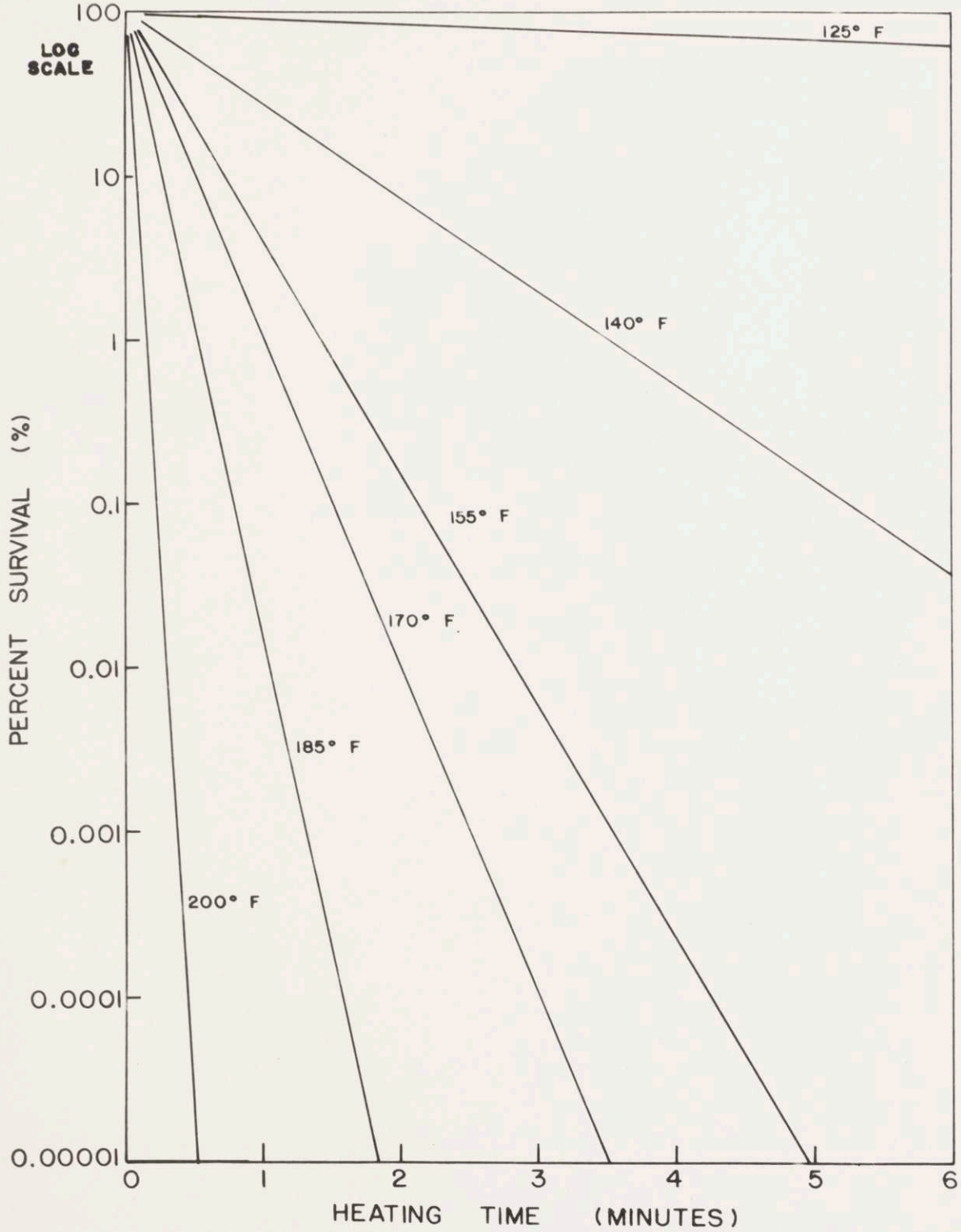

Figure 7. Composite of Survival Curves at 125° to 200° F of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated in Capillary Tubes.

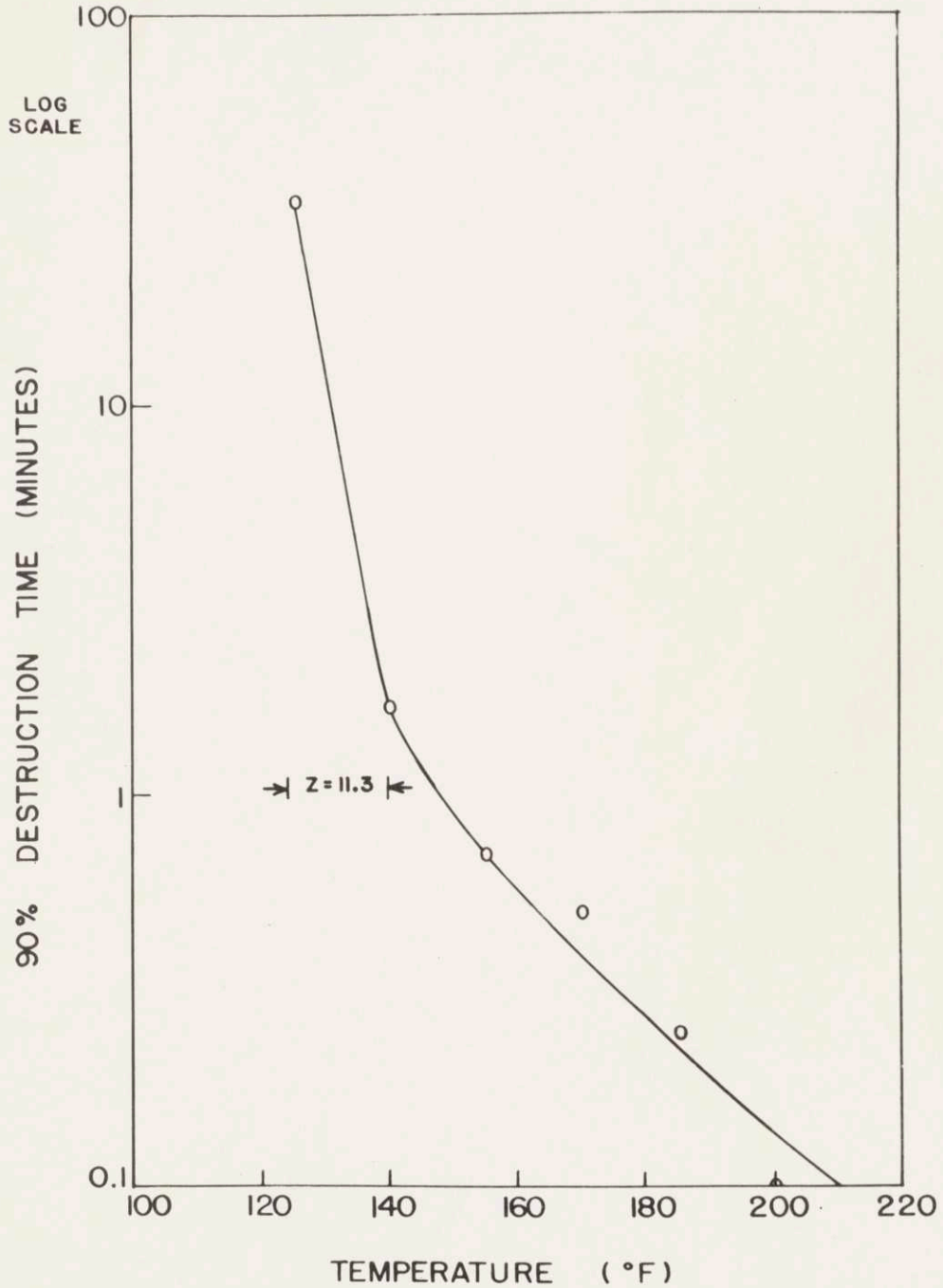

Figure 8. A Thermal Death Time Curve Based on D Values of Micrococcus pyogenes, var. aureus, F.D.A. 209 Suspended in Ham Homogenate (13% Solids) Heated in Capillary Tubes.

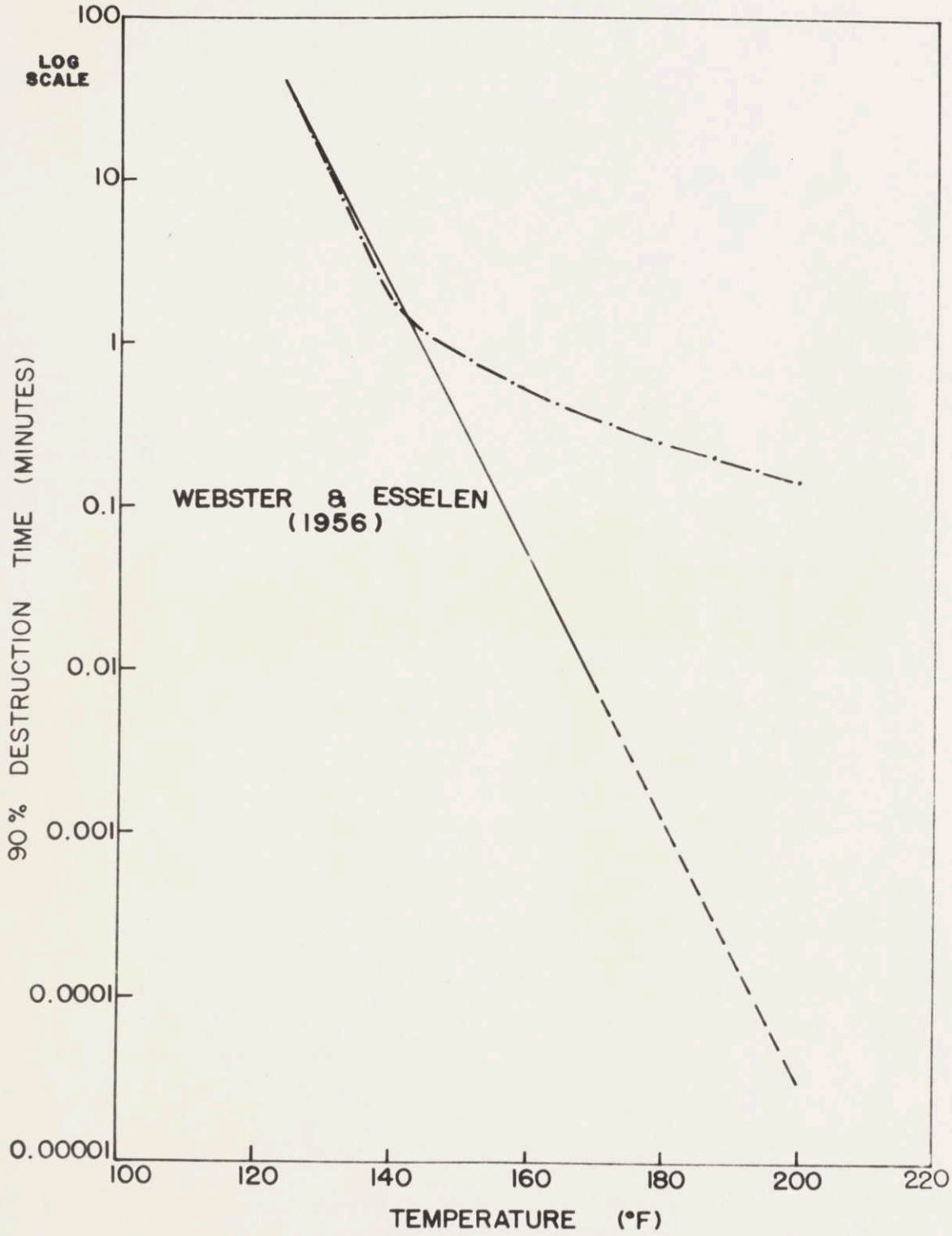

Figure 9. Two Thermal Death Time Curves of M. pyogenes, var. aureus Drawn from D. Values. That of Webster and Esselen is Extrapolated to 200° F; the Other was Determined in this Investigation (Also Shown in Figure 8).

C. THEORETICAL DISCUSSION OF THERMAL DEATH TIME DATA

The thermal death time data presented in the previous section should be carefully considered. The divergence of the thermal death time curve from accepted values at higher temperatures requires explanation.

One possible reason for these results could be that the method is in error. This error could be either inherent within the basic procedure, or it could be the result of some consistent experimental error. The break in the thermal death time curve is shown to occur at 140°F, and there is only one lower temperature (125°F) below this point which, if in error, could greatly influence the curve. The argument against the idea of an experimental error, so severe to sharply alter the thermal death time curve, lies in the fact that the method produced results consistent with those found in the literature at lower temperatures.

If it is assumed that the method is correct, actual occurrences at the higher temperatures might be considered. Logic could be employed to show that a finite period of time is required for the transfer of heat energy to the

sensitive areas of the cell, and still a further increment of time passes before the cell is affected.

Logic may also be applied to show that the lag of the lethal effect is, again referring to the inherent error concept, a function that occurs only at higher temperatures. Perhaps these temperatures do not allow the material within the capillary tube to come to equilibrium during the short time of heating.

There may be an explanation which can be used to prove the correctness of the thermal death time curve, derived in this work. If such can be proved, then perhaps the mechanism of action can be better understood.

This possible explanation lies in the kinetics of the reaction which results in cellular lethality as has been suggested in the literature. It is based on the Arrhenius Equation:

$$k = Se^{\frac{-E}{RT}} \qquad (1)$$

where S is a frequency factor, describing the frequency with which molecules in a chemical reaction collide and exchange energy, E is the energy of activation required for the reaction to proceed, R is a constant, and T is absolute temperature.

The lethal reaction at a given temperature depends on both the energy required to cause the reaction and

the number of collisions per unit time. Charm (1957) developed a concept describing this. Within the bacterial cell, there are many "sensitive volumes". Inactivation depends on energy at a given level (E) reaching these volumes. The volumes are in an aqueous phase, surrounded by many water molecules. When a water molecule has enough energy, and is in contact with the sensitive volume, inactivation occurs. Energy at a given temperature will be distributed in gradient levels throughout the water molecules involved. There is, therefore, a given fraction which can cause lethality, based on a sufficient energy level being reached. In addition, these molecules are in constant vibrational motion, quantitated by S. Reactions resulting in lethality are then dependent upon the water molecules, possessing energy E, striking a sensitive volume within the cell.

The relationship between the logarithmic survival curve and the kinetic approach has been shown by Gillespy (1951), Pfeifer and Vojnovich (1952), and Charm (1957). This has been carried out by the use of the concepts of chemical kinetics to derive the equation describing the logarithmic survival curve, and thence, the thermal death time curve.

The relationship between chemical kinetics and bacterial response to heat can be described. In a chemical reaction, the reactants do not all react at once although there is reasonable assumption that there is no difference between molecules. Because there is a finite period through which the reaction proceeds, there are forces involved in the basic nature of the reaction which influence this distribution of reactants. The Arrhenius Equation shows that three variables are responsible, the frequency of collisions of reactants, the energy of activation, and the temperature of the reaction. Temperature is less important in this discussion than the other two factors. It is a measure of the median energy of the reactants, but does not describe the forces involved, merely the energy state at which the action proceeds.

In a similar sense, bacterial cells are affected at a given rate by what is considered to be a protein denaturization. Again, the reaction may be dependent upon the frequency with which water molecules, at an energy level sufficient to cause the lethal reaction, collide with sensitive reactants to transfer this energy. With increased temperature, the energy levels of the water molecules are increased and the movement of the water molecules becomes more rapid. Both of these

factors decrease the time required for the lethal reaction to proceed.

The data for the thermal response of Micrococcus pyogenes, var. aureus in ham solids at a temperature range of 125° to 200°F may be treated by the concepts of chemical kinetics. In the previous section, treatment of data followed the conventional concepts.

The energy of activation and frequency factor S may be determined from the Arrhenius Equation (1) in the following manner:

The constant k in the kinetic equation is the rate constant. It is also the death rate constant for the logarithmic death of bacteria, (Perkins, 1954),

$$k = 1/t \log \frac{\text{initial number}}{\text{number of survivors}} \qquad (2)$$

where t represents heating time in minutes.

To destroy 90 per cent of the initial concentration of cells (n_o),

$$k = 1/t \log \frac{n_o}{.1\ n} \qquad (3)$$

Or,

$$k = 1/t \log 10 \qquad (4)$$

As log 10 equals 1, and the time to destroy 90 per cent is the D value

$$k = \frac{2.303}{D} \qquad (5)$$

By plotting log k against the reciprocal of the absolute temperature, a line of slope $\frac{-E}{2.303R}$ may be drawn. E is calculated from this. Knowing E, it is then possible to calculate the frequency factor S through a range of temperatures.

Calculations are based on absolute temperature (°F + 460). A value of 2 Btu/°R-cell (Charm) was used as a value of R. Energy of activation was calculated on a basis of Btu/cell.

Using the data given in the previous section, the following table was set up:

°F	°R	$\frac{1}{°R}$	D	$k = \frac{2.303}{D}$
125	585	0.00171	36.25	0.635
140	600	.00167	1.74	1.32
155	615	.00163	0.73	3.15
170	630	.00159	0.51	4.52
185	645	.00155	0.25	9.20
200	660	.00151	0.10	23.03

The plot of log k against 1/°F, based on these data, is shown in Figure 10.

The nature of the curve is of interest, for it is not linear over the entire temperature range. The

segment between 0.00171 and .00167, corresponding to heating at 125° and 140°F, respectively, differs greatly from the remainder of the values, which have a linear relationship.

The same graph shows similar treatment of the data of Webster and Esseln (1956). The use of these data is based on the substantial survival data presented. They used the plate count method for survival, which has been previously described as being probably more accurate than the culture tube method in which growth or non-growth is recorded. The results of the data from the previous section follow the trends of the data derived through the temperature range of 125° to 140° used by Webster and Esseln. They extrapolated their thermal death time curve to 168°F, and reported results at 165°F.

For this kinetic study, Webster and Esseln's curve has been further extrapolated to 200°F for comparison purposes; they made no statements about lethality beyond 165°F.

The following table shows the treatment of data used to construct the curve.

°F	°R	$\frac{1}{T_R}$	D	$k = \frac{2.303}{D}$
125	585	0.00171	36.0	0.0639
140	600	.00167	2.20	1.045
155	615	.00163	0.14	16.43
179	630	.00159	0.0080	287
185	645	.00155	0.00047	4890
200	660	.00151	.000032	71800

(From data of Webster and Esseln, 1956)

When their data is treated kinetically, a straight line is obtained through the entire temperature range. The slope of the line is $\frac{-E}{2.3R}$, from which the energy of activation is calculated to be 321,000 Btu per cell.

For the data of this work, E is calculated, in the same range between 125° and 140°F, as 311,000 Btu per cell. Through the remaining five points, from 140° to 200°F, the energy of activation is calculated to be 75,000 Btu per cell. The value of E for the Webster and Esseln curve, however, does not change.

From the values of E, the frequency factor S may be found by the use of the Arrhenius Equation (1). The natural logarithm is

$$\ln k = \ln S - \frac{E}{RT} \qquad (6)$$

converting to common logarithms,

$$2.3 \quad \log k = 2.3 \log S - \frac{E}{RT} \qquad (7)$$

and,

$$\log S = \log k \neq \frac{E}{2.3RT} \qquad (8)$$

The calculated values of S are shown in the following table:

Temperature (°F)	Value of S (1/minute) This Data	Webster and Esseln
125 - 140	1.32×10^{113}*	1.05×10^{116}
140 - 155	1.58×10^{27}	1.64×10^{115}
155 - 170	5.68×10^{27}	2.87×10^{112}
170 - 185	2.91×10^{26}	4.89×10^{111}
185 - 200	1.83×10^{26}	7.18×10^{106}

* Based on E equal to 311,000 Btu per cell.

The value of 75,000 Btu for the activation energy is in the same range of magnitude as that calculated by Charm. He found that Cl. Sporogenes, P.A. 3679 has a value of approximately 56,000 Btu in five different media. Cl. botulinum was found to have an activation energy of approximately 66,000 Btu per cell in three media.

Charm found the frequency factor to vary slightly with temperature and medium. For P.A. 3679, S ranged from

1×10^{17} to 6×10^{18}, and a range of 2×10^{20} to 4×10^{22} was shown for Cl. botulinum.

The results of the data from this work above 140°F seem to be of the same order as those obtained by Charm. The value of the energy of activation is somewhat higher. The significance of these differences in value cannot be established. There are three factors that make direct comparisons difficult. These are the species variation from anaerobic spores to vegetative cells, the differences in media, and the range of temperatures. Any significance could only be evaluated by a background of data from the literature on the response of vegetative cells to higher temperatures, and on further work using the kinetic theory.

If the results of the work done in the previous section are to be taken as correct, it would seem that there are factors associated with heating cells of Micrococcus pyogenes, var. aureus at temperatures above 140°F which are obscured or modified by the effects of heating at temperatures below 140°F. This might account for the divergence of results. Some insight into the problem may well be gained by considering what would happen below 125°F. Extrapolation through another 15° increment would reach a point about 10° above normal incubation temperature. At 110°F, or lower, lethal processes proceed much more

slowly and growth occurs. Extrapolation of the thermal death time curve shows that it would require 10 hours at 110°F to reduce the population by 90 per cent, and 160 hours at 100°F.

It is unlikely that these values can be derived with any validity in this fashion, but it does show an interesting point. The death of cells at these temperatures is due to much more than just a thermal effect. Such factors, as normal death,buildup of inhibiting metabolic products, lack of nutrients in the environment, and growth rates are involved. It is probable that the lethality associated with these temperatures is not necessarily a direct function of effects due to heating.

The existence of lethal factors from causes other than heat does not stop at a given temperature, to be assumed only by the lethality of heat as temperatures increase. In all probability, the transition is a gradual one, beginning somewhat above the incubation temperature of 100°F. If any of these influences or other factors are involved with death in the temperature range of 125° to 140°F, then the variation in results may be explained.

However, as discussed earlier and in Part B of this section, it is unlikely that the same relationships of

bacterial death hold at the extremes of the temperature range.

It is not claimed that this discussion proves the validity of the curved thermal response above 140°F. Its purpose is merely to show that such a response is likely, and that there may be a certain error involved in the extrapolation of thermal death results beyond the temperatures actually employed.

This whole area of study needs considerable investigation. This work may be forthcoming with the advent of concepts of lethality at higher temperature, both those dealing with sterilization and with pasteurization.

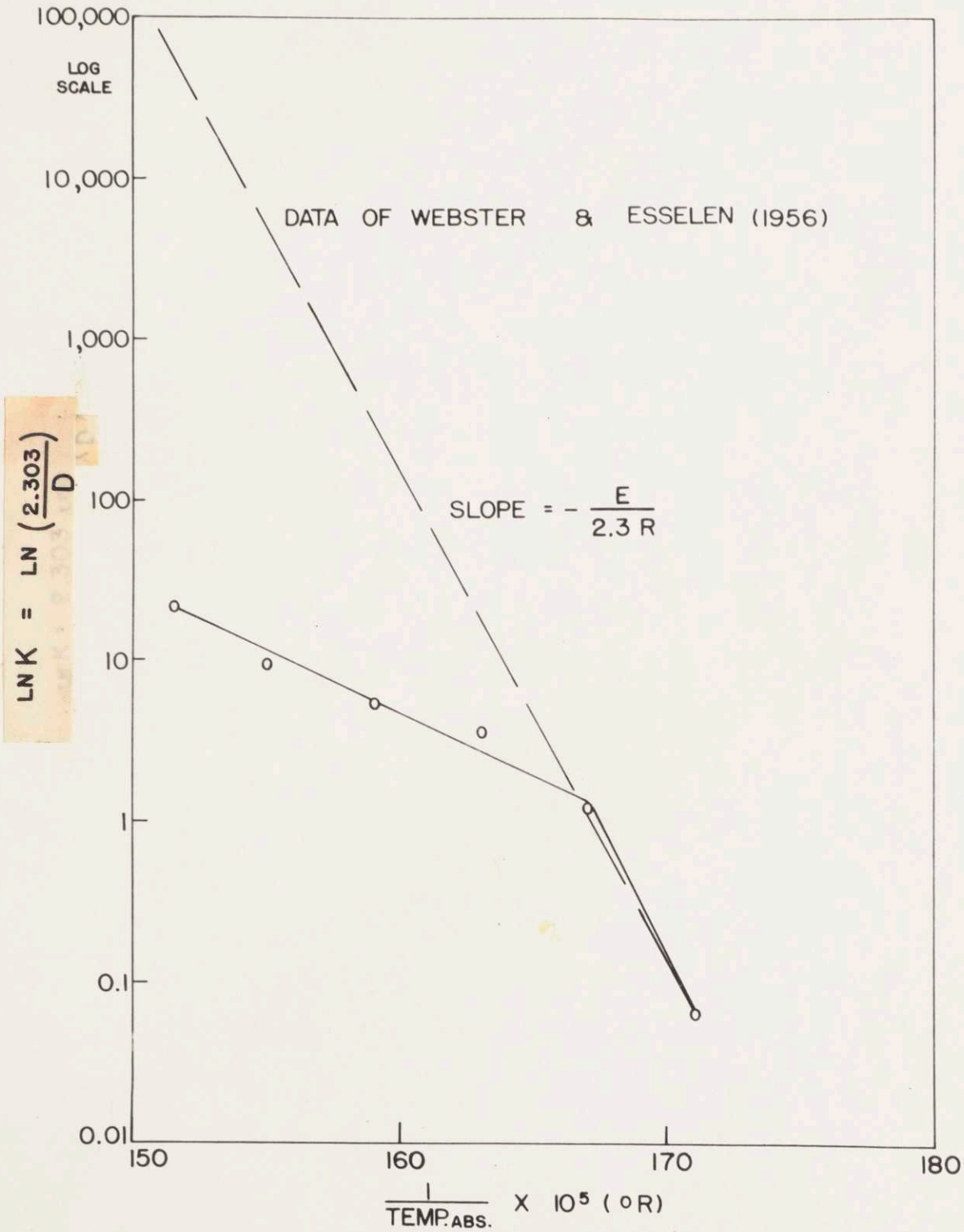

Figure 10. Graph Based on Thermal Death Time Data from the Curves of Figure 9 of M. pyogenes, var. aureus Drawn for Determination of Energy of Activation Values from the Slopes.

IVc. THE DESTRUCTION OF AN ENTEROTOXIN PRODUCING STRAIN OF MICROCOCCUS PYOGENES, VAR. AUREUS AND RESIDENT FLORA IN BOILED HAM AND ROAST BEEF SLICES BY AIR OVEN HEATING

A. EXPERIMENTAL PROCEDURE

1. Heating Equipment

A Despach Oven with a rotary stone hearth was used for heating. This oven has a high heat capacity, and was designed for test baking. Heating coils at the top and bottom supply radiant heat to the hearth. The oven is insulated, except for a small hole in the top to enable vapor to escape. The door is small to minimize the heat loss when open.

The oven is equipped with automatic controls. For this work, it was considered that the controls might not be sufficiently accurate for close temperature control. To achieve a finer control of temperature, the controls were set for an approximate temperature, and the exact temperature was obtained by further manipulation of the controls or by opening the oven door.

Temperature was measured by copper-constantan thermocouples. The temperature-sensing junction was secured to the surface of a small slice of meat, which was wrapped with single thickness of heavy gauge aluminum foil. This arrangement provided accurate measurement of the temperature at the surface of the slice. This temperature was critical because it was the temperature at which the microorganisms were being destroyed. Another thermocouple was laid on the hearth and secured to provide contact with the surface of the hearth, while another was suspended in the air inside the oven.

2. Preparation of Samples

Roast beef and boiled ham were obtained and sliced as needed with a Hobart, motor-driven meat slicer. A constant setting of No. 9 on the slicer produced a slice of 2.1 ml thickness, with a range of 1.6 to 2.7 ml. The pieces were trimmed before being sliced only to remove external excess fat. The meat was sliced "as is" to similate commercial practice. The face slices were discarded because it was considered that the microbial flora present might differ significantly from that of succeeding slices.

Slices were cut into 22 gram portions. This weight was selected for the purpose of having the correct weight

to provide a dilution of 1:10 by the addition of 198 ml of sterile distilled water. The area of 22 gram slices was approximately 10 sq. in., roughly that of a slice of bread.

After being weighed, the slices were placed on a sheet of fresh aluminum foil, which was folded over to provide a loose cover for the slices, reducing excess air-borne contamination.

Inoculation was considered by pipetting 2.5 ml of a 24 hour culture of Micrococcus pyogenes, var. aureus with a 10 ml graduated, sterile pipet. The inoculum was placed drop-wise over the entire surface of each slice. The aluminum foil cover was folded over, and the slices were allowed to stand for 30 minutes. At the end of this period, the slices were turned over to ensure complete distribution of the inoculum by inverting the aluminum foil so that the cover was then the bottom. The slices were allowed to stand for another 30 minute period. The total time of one hour was sufficient to allow the culture to be well distributed throughout the slices, and to allow excess moisture from the inoculating medium to evaporate.

The inoculated slices were then packaged in aluminum foil envelopes, as described previously in Section III-B-2, and were ready for processing.

3. Description of Organism

The strain of Micrococcus pyogenes, var. aureus used for inoculation was coagulase positive and an enterotoxin producing type. It was isolated after a serious outbreak of food poisoning in the summer of 1956 from ham, which was used in sandwiches. The outbreak occurred in a summer camp, with virtually all members being stricken. It was a high pigment producer when incubated at 37°C.

4. Processing of Slices

One hour before processing, the oven temperature was determined with the three thermocouples. The exact processing temperature was obtained either by manipulation of the heating controls to increase temperature, or by opening the door to allow heat to escape with a corresponding lowering of the temperature control. These changes were slight. The oven was used almost exclusively for this work, and thus after a few trials, sufficiently accurate control was possible with a minimum of changes. The oven was allowed to warm to the desired temperature for about twelve hours before processing was carried out. When the desired conditions of temperature were obtained, i.e., the three thermocouples gave similar readings, indicating equilibrium,

the controls and the door were not touched until processing, so that the desired temperature was maintained.

The conditions of processing were:

Temperature	Time of Processing (minutes)
185°F	0, 2.5, 5, 7.5, 12.5, 15
200°F	0, 2, 4, 6, 8, 12
250°F	0, 1, 2, 3, 4, 5
300°F	0, 1, 2, 3, 4

To minimize heat loss due to opening the door to remove and introduce samples, only one sample was heated at a time. When it was removed, another sample was introduced. After being placed in the oven, the hearth was turned through 180° so that the slice would be at the back of the oven away from the door. The opening of the door was reflected in a sharp drop in air temperature, which recovered within 30 seconds. There was little or no change in the hearth temperature or the temperature at the sensing element which was covered with aluminum foil.

Immediately after processing, the samples were removed to a refrigerator at 38°F, where they were held until plated.

Duplicate slices were processed for purposes of preparing samples for freezing.

5. Counting of Treated Samples

Samples were prepared for counting by being removed from the package and blended with 198 ml sterile distilled water in a Waring Blendor. Removal from the package was carried out by tearing the folded edge along three sides of the envelope and dropping the slices into the cup. Samples were blended for two minutes at high speed, after which dilutions were made, and were plated in duplicate.

Counts were made in accordance with Standard Methods for the Examination of Dairy Products (1953) using Tryptone Glucose Extract Agar. Cultures were incubated at 37°C.

6. Treatment of Data

Duplicate counts were averaged for each slice. The counts were calculated and expressed on a one gram basis. The counts of unheated controls were used to represent 100 per cent survival. In calculations of survival, this mean count was used as the divisor. The calculated "percent survival" values were plotted logarithmically against corresponding temperatures to produce survival curves. Regression analysis was used in the determination of the "best straight line".

B. RESULTS AND DISCUSSION

The curves of Figure 11 show the survival of Micrococcus pyogenes, var. aureus in ham slices during heating at various temperatures; Figure 12 show the survival in roast beef slices.

The important point to be considered is that these slices were not sterile before inoculation. The goal of this work was to parallel commercial practice. Slices were prepared under the conditions of cleanliness that might be expected under operating conditions. If meat containing Micrococci was sliced, possibly contaminated by a handler, and allowed to stand for some hours prior to use, large counts up to the magnitude of the inoculum that contained approximately 50×10^6 organisms might be expected.

The effectiveness of a heat processing program should be measured by its ability not only to reduce inoculated flora to safe levels, but also to remove any resident flora from the meat slice. These microbiological effects are evaluated in this section.

In table, the following data are given:

a. Lethality Lag (time of first bacterial destruction).

b. Slope of Survival Curve.

c. Time Required to Obtain a 90% Reduction of Viable Cells.

d. Time Required to Obtain a 99.99999% Reduction of Viable Cells.

The data were taken from the survival curves.

SURVIVAL DATA OF UNSTERILIZED MEAT SLICES INOCULATED WITH AN ENTEROTOXIN PRODUCING STRAIN OF MICROCOCCUS PYOGENES, VAR. AUREUS

Heating Temp. (°F)	Lethality Lag (minutes)	Slope of Survival Curve	Time for Reduction of Population (minutes) 90%	99.99999% *
HAM				
185	3.6	2.4	6.0	20.5
200	1.1	1.3	2.4	10.3
250	1.2	0.4	1.6	3.9
300	0	0.7	0.7	4.8
ROAST BEEF				
185	3.4	3.2	6.7	25.8
200	1.6	1.8	3.4	14.3
250	1.0	0.6	2.7	5.6
300	0.1	0.7	0.8	5.0

* Plotted in Figure 13.

The lethality lag, measured in minutes, is the result of two factors. The first is the time required for heat

transfer through the package and penetration into the slice. This heating inertia would increase with increased packaging thickness, thickness of slice, and lack of contact between the package and slice. The latter is the difference between conduction transfer of heat between the meat and the package, and heating through an air space which would provide for conductivity. This is one advantage of a close-fitting, envelope-type package.

The slope of the survival curve, defined as the time required to transverse one logarithmic cycle of survival, is shown to be a direct function of temperature.

The reduction time of 90 per cent of the initial population has associated with it the initial lag in lethality, which is included to give a general idea of the total time required to achieve most of the lethality. In most work of this nature, a correction for zero time is made. However, this work was designed with commercial practices in mind, and the concept of total heating time must be known in order to set up process specifications. The lag preceding the lethal effect was found for each temperature, and is shown in the previous table.

The time required to destroy 99.99999% of the organisms was arbitrarily taken as the measure of pasteurization effectiveness. It is the time required to reduce a given population of 1×10^7 cells per gram

to a final concentration of one cell per gram. The use of percentages of survival in evaluating this destruction is advantageous because minor variation in initial plate counts is not considered in calculating percentages. As shown in the graphs, this end point is the time required to transverse seven logarithmic cycles of survival.

The results show that there is little difference in the results obtained after heating at 250° and 300°F. Approximately twice the heating time is required at 200°F, and over four times as much at 185°F. These data have been compiled, and plotted in Figure 13. These two curves for each of the substrates show how processing time is a function of temperature, and also enables the determination of the time required at any intermediate temperature to attain the same effect.

The abrupt change of slope in the range of 200°F is of great interest. The reason for this is based on the overall thermal resistance of Micrococci pyogenes, var. aureus. Although it has high resistance relative to the vegetative group, this resistance is overcome by the temperatures above this range. Its resistance is also much lower than that of the sporulating group. The resident organisms in the meat before inoculation include both resistant spores and vegetative cells, but the inoculum accounts for by far the greatest fraction.

The vegetative cells, having a low resistance to heat, are rapidly destroyed at temperatures up to 200°F, at which point most of the Micrococci are also destroyed. The D value calculated in Part b was shown to be 0.10 minutes at 200°F, which shows the high order of lethality at this temperature. Above 200°F, lethality is based on any surviving organisms, which either have been protected in some way by the meat or have a particularly high resistance. Lethality of the spores present is probably of less importance in this work. The anaerobic spores, Clostridium species, will not grow on T.G.E. plates under the conditions used in preparing the cultures, but aerobic spores might grow, or if protected during the early heating stages, might germinate. These Bacilli might only slightly influence the survival curves.

It is shown that the bacteria have a consistently slightly greater lethality in ham. The curves for the two substrates roughly parallel each other. This difference between the two meats would probably be neglected in incorporating safety factors into commercial process specifications of time and temperature. Such factors would include an overall percentage increase over the time required to obtain adequate destruction. This allowance would be necessary because of the heat loss due to the heat capacity of the unprocessed meat at its

lower initial temperature, if large amounts were to be processed relative to oven capacity; losses of oven heat during introduction and removal of product, if done on a continuous basis; and finally, allowance would be made for the lethality during cooling. This last factor was reduced substantially in this work by immediate refrigeration after processing.

It is difficult to give exact reasons for the differences in response between organisms in the two substrates. It may be due to some physical factor, such as the rates of heat transfer within the meat or interstices within the fibers, or the protective effect of fat or other factors. On the other hand, the differences may be accounted for by chemical factors, especially the presence of curing compounds within the ham, or changes in the chemical character of the substrate resulting from the boiling of ham after curing. The differences are not great, but are consistent.

It is interesting to note that the curve in Figure 13 for roast beef slices can be drawn through each point, whereas that for ham cannot be drawn through the points to provide a smooth curve. This may just be an artifact, or may be due to a variation between samples of ham. The roast beef was obtained weekly from a

restaurant where the purchaser went to great lengths to obtain uniform, top of the round, choice quality beef. The pieces selected for this work showed remarkable uniformity of grain, degree of cooking, and fat. Ham, on the other hand, was purchased locally, and showed a much greater variation in grain, fat, and connective tissue areas. Within one piece of ham, great variations could be expected. This may account for the variation, if the relative lack of uniformity resulted in some bacterial effect.

The conditions of processing were not deteriorative to either of the products at any of the times and temperatures used. Ham actually improved in flavor, assuming a more baked-like taste at the higher temperatures. The limiting quality factor would be the rendering of fat and water. This did not appreciably lower the quality. Although some fat and water came to the surface during the heating, and was found in the inside of the foil package when opened, this was not much more excessive than the fat and water found with unheated samples held several days in foil packages.

It is considered that this work substantiates the premise that far more efficient bacterial destruction may be obtained by heat processing meat slices rather than by processing large pieces of meat. The success

of this method could well be incorporated into menu preparation. If operational conditions permit, it is conceivable that processed meat slices could be issued together with a package of two bread slices. The crew of a plane, to continue with Air Force application, could fabricate the sandwich just before eating. The slice would not be subject to handling during the period after processing until consumption. After issue, the slice would be held at ambient temperatures, permitting possible growth of organisms surviving the processing operation. Growth to hazardous levels would require time in excess of the five hours now allowed because the initial concentration would be so low. This seems to be a practical solution. The expense of such a processing scheme might be more than justified if these items were centrally processed, and the costly operation of 24 hour in-flight kitchens were reduced. These kitchens could then have the function of storing and issuing menus, instead of the task of complete fabrication as close as possible to flight time.

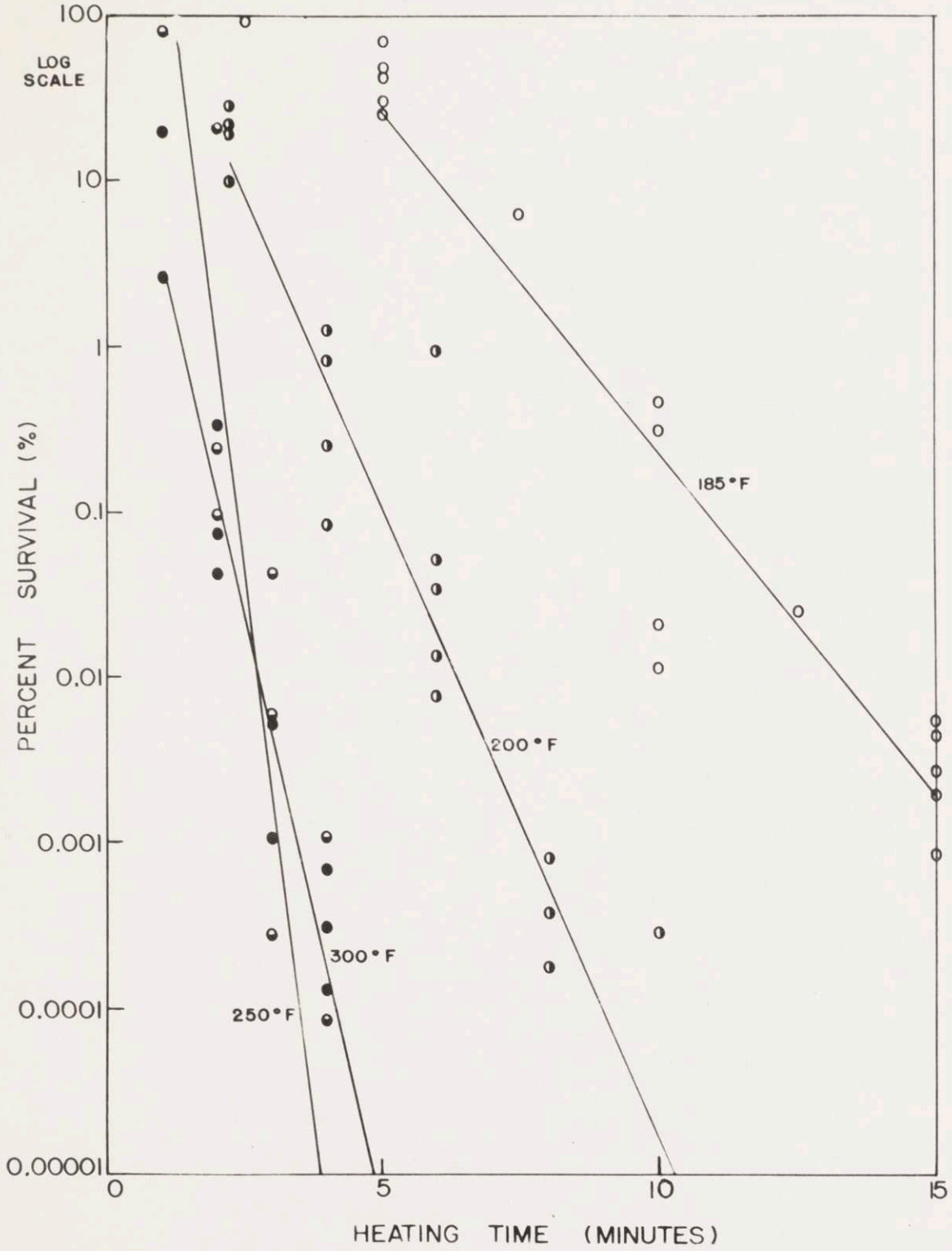

Figure 11. Survival Curves of an Inoculated Enterotoxin Producing Strain of Micrococcus pyogenes, var. aureus and Flora in Boiled Ham Slices Heated in a Hot Air Oven.

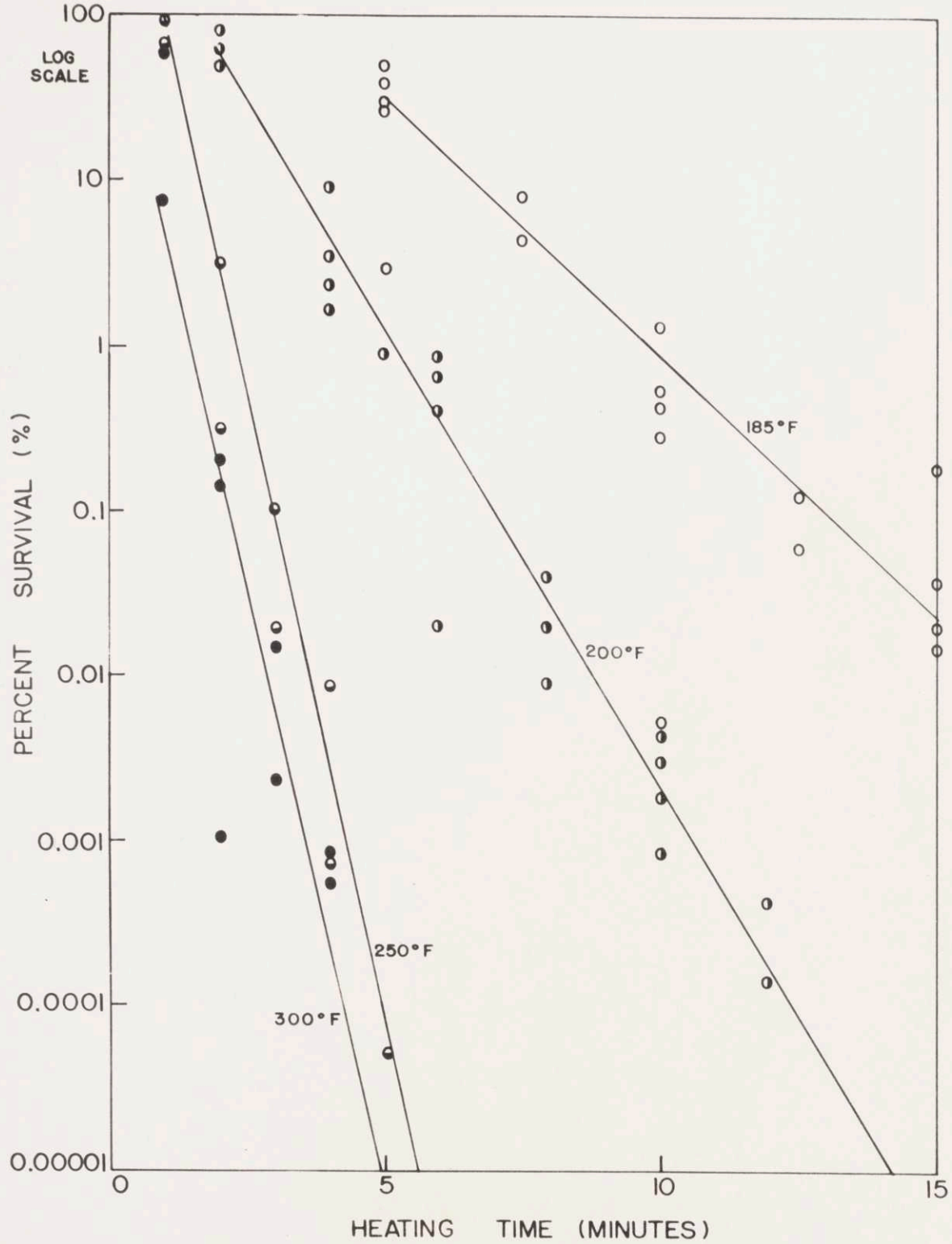

Figure 12. Survival Curves of an Inoculated Enterotoxin Producing Strain of Micrococcus pyogenes, var. aureus and Flora in Roast Beef Slices Heated in a Hot Air Oven.

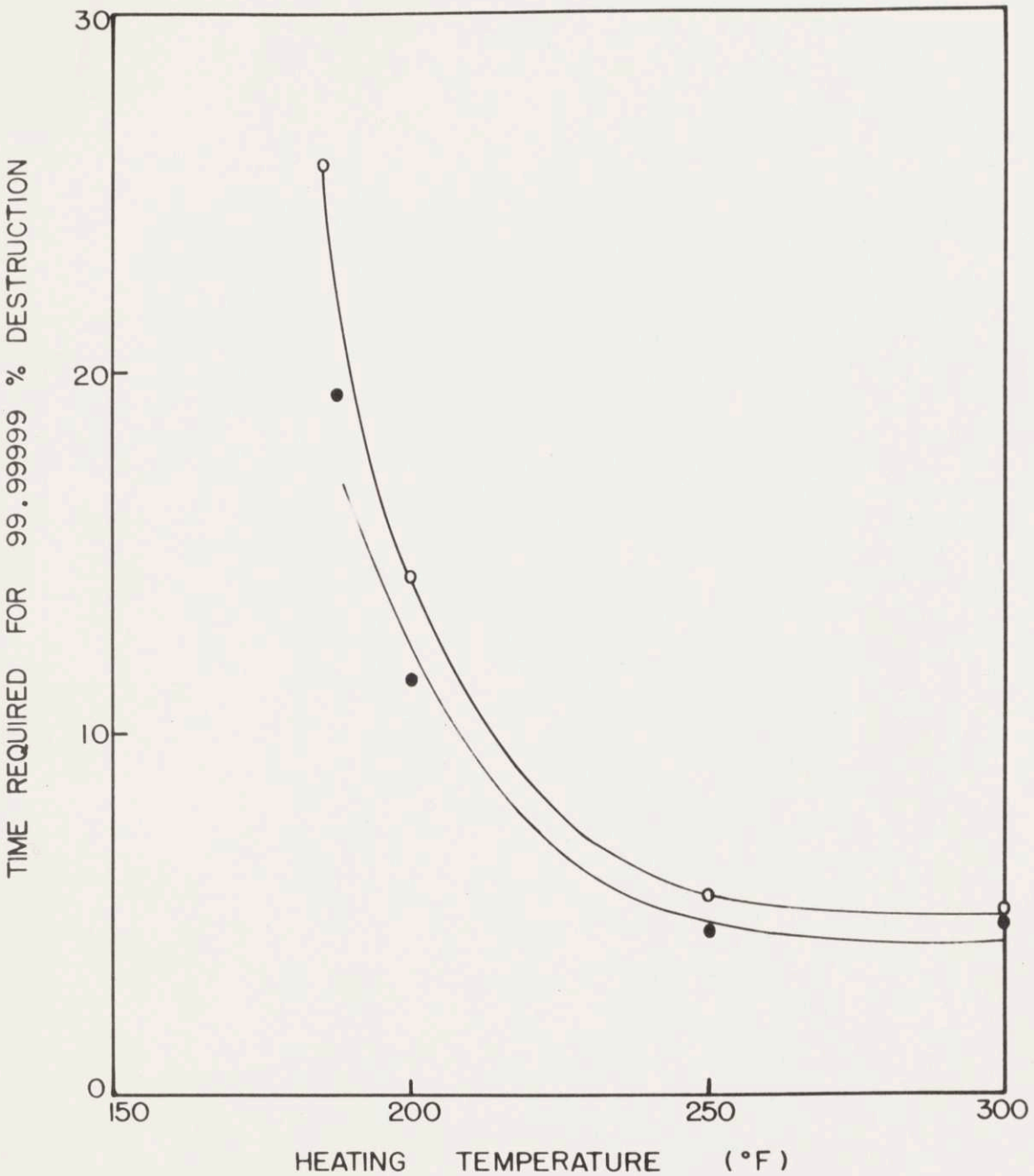

Figure 13. Time-Temperature Relationships for the Destruction of an Inoculated Enterotoxin Producing Strain of M. pyogenes, var. aureus and Flora in Meat Slices Heated in a Hot Air Oven.

IVd. THE RELATIVE HEAT RESISTANCE OF THE TWO STRAINS OF MICROCOCCUS PYOGENES, VAR. AUREUS USED IN THIS WORK

1. Introduction

Two strains of Micrococcus pyogenes, var. aureus were used in this work. One strain was an enterotoxic-negative and coagulase-negative organism, known as F.D.A. 209, ATCC No. 6538. This strain is widely used as an indicator organism. It is used as a standard in the Phenol Coefficient Method, the penicillin assay, and is frequently used in assays of other antibiotics.

The other strain used was an extremely active enterotoxin producing strain of Micrococcus pyogenes, var. aureus. It was the cause of a severe food poisoning case at a summer camp in Massachusetts in 1956, and was isolated from ham sandwiches by the State Public Health authorities.

In this work, for highly controlled tests, the 209 strain was used. These tests involved techniques free from contamination. The object of these examinations was the exact measurement of the organism's response to certain processing conditions. Because it formed the

basis for applications work, it was considered that a standard indicator organism should be used. In this way, any of this work could be repeated with the same organism. Further basic work could be continued with such a standard and well-known strain. This organism was used in the following experiments:

The Determination of Thermal Death Rates	Section IVb
The Influence of Chemicals and Antibiotics on Heat Resistance	Section V

For applications work, it was considered advantageous to use the enterotoxin producing strain. The meat slices used contained normal flora and contaminants from handling. The feasibility of using a pathogenic microorganism for this work was considered important. The bacterial effect of processing was designed to attain the destruction of large numbers of an enterotoxin producing strain present with the normal flora.

The exact response of the organism under such conditions could not easily be measured. The variation of the indigenous flora, thickness of meat slices, and inexact methods for precise control of heating and cooling make lethal rates less precise than those obtained using the capillary tube method. The enterotoxin producing organism was used in the following cases:

Since two bacterial species were used, the necessity arose to relate the individual responses of each. Slight differences would be of little importance because they would be overshadowed by the varying and perhaps greater influences due to the method of processing used for meat slices. Large differences would be important, however, because the use of one strain for the basic work and another for the processing would not be valid.

2. Methods

The methods used to evaluate the individual responses to heat of the two strains are those developed for the measurement of antibiotic and chemical influence on the thermal death rate, and are fully described in the following Section, V-A.

The procedure involved the suspension of large numbers of cells of pure cultures in sterile ham and roast beef homogenates. The samples had a volume of 2.5 ml, and were heated for 0,5,10,15,20,25 or 30 minute intervals at 140°F. Survival curves were obtained, and compared.

3. Results and Discussion

The curves in Figures 14 and 15 show the survival of the enterotoxic producing strain. Figures 16 and 29 show the survival curves of strain 209, which were used as standard curves for the work with the chemical and antibiotic agents in Section V.

The data from these curves were used as sample calculations to show the linear regression method. They are shown in the Appendix, Section G, with data shown in Section C.

Regression analysis of the curves showed no significant difference between the two organisms suspended in either ham or roast beef solids. There also was no difference between the effects of the two substrates.

Although this method does not have the extreme accuracy of the capillary tube method for the establishment of thermal lethal rates, its level of accuracy is much higher than that which would be obtained if the processing of meat slices were used to detect differences. It is possible, however, to find relative differences by this procedure. As no differences were found by this method, it certainly indicates that no differences would be expected with the less sensitive methods.

The lack of a significant difference between the two strains may be quantitated further. As was shown in the sample calculations for tests of significance, results obtained in ham substrate show that comparison of regression lines of the two strains has a t value of 0.677. The 95 per cent confidence level for the same degree of freedom is represented by a t value of 2.306. Roast beef, although not shown in the sample calculations, had a value of the same order as ham.

For this reason, it is considered that the strains would show similar responses to heat in a processing application, where any slight differences would not be detected. The premise of using an extremely well known strain for basic work, making possible substantiation of results or continuation of work, is valid. Similarly the use of an enterotoxic strain for application work is valid, and is desirable because it more closely simulates commercial operations, which have the destruction of food poisoning strains as their goal.

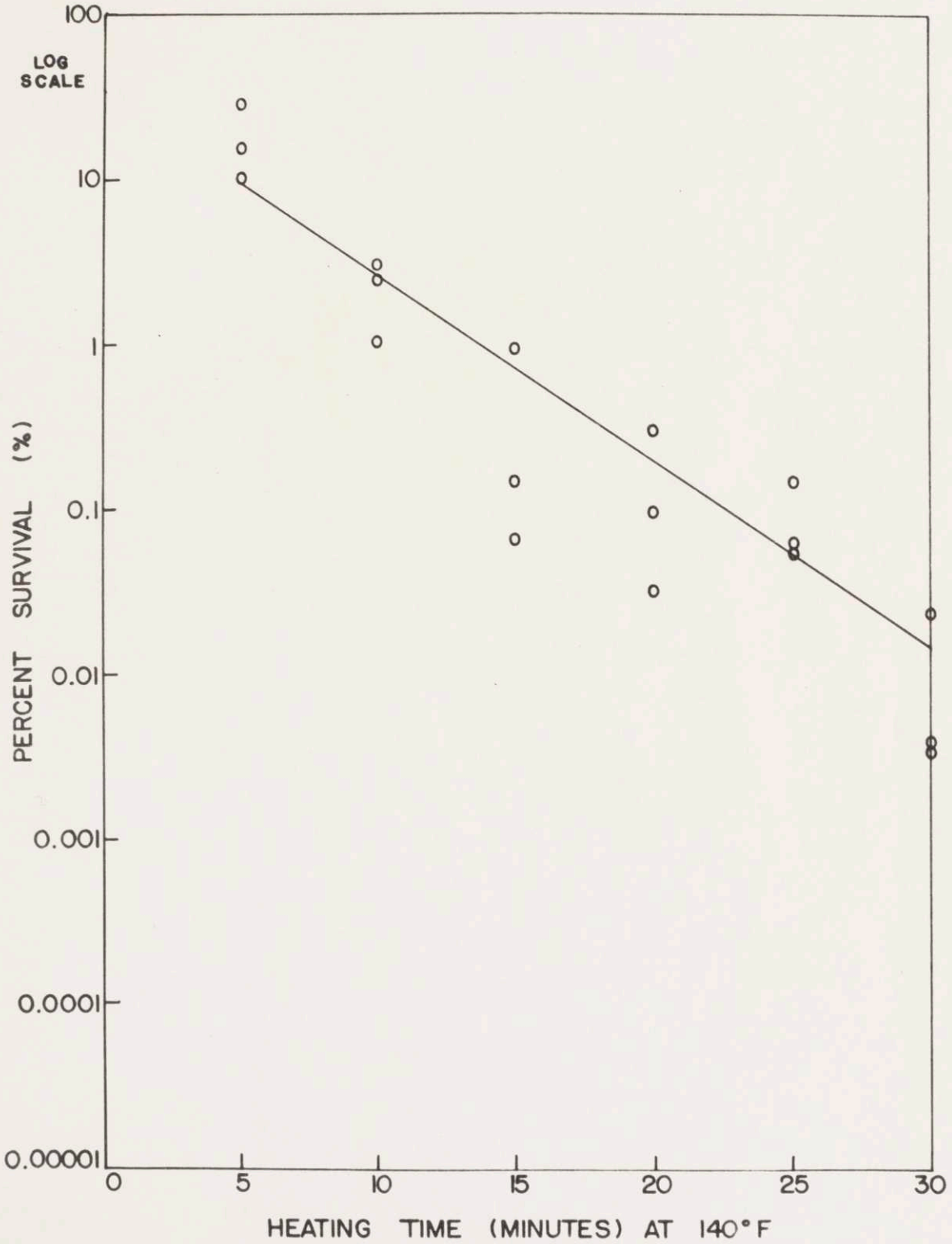

Figure 14. Survival Curve of an Enterotoxin Producing Strain of M. pyogenes, var. aureus Suspended in 2.5 ml of Ham Homogenate Heated at 140° F.

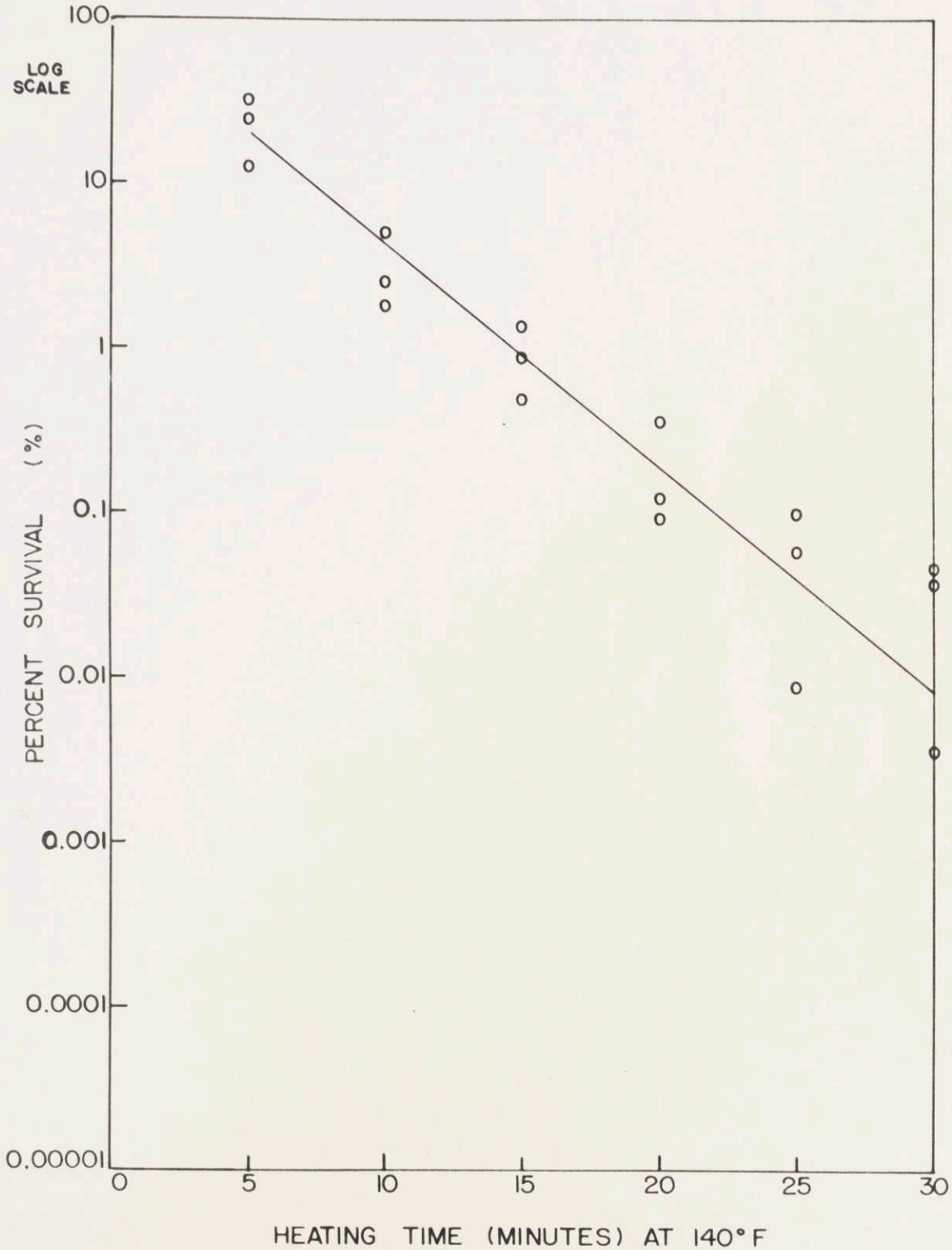

Figure 15. Survival Curve of an Enterotoxin Producing Strain of M. pyogenes, var. aureus Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F.

V.

INFLUENCE OF CHEMICAL AND ANTIBIOTIC SUBSTANCES ON THE HEAT RESISTANCE OF MICROCOCCUS PYOGENES, VAR. AUREUS SUSPENDED IN ROAST BEEF AND BOILED HAM HOMOGENATES

V. INFLUENCE OF CHEMICAL AND ANTIBIOTIC SUBSTANCES ON THE HEAT RESISTANCE OF MICROCOCCUS PYOGENES, VAR. AUREUS SUSPENDED IN ROAST BEEF AND BOILED HAM HOMOGENATES

A. EXPERIMENTAL PROCEDURE

1. Development of Method

A method was sought which would permit rapid and accurate determination of any change in heat resistance affected by the presence of a chemical material in the medium. The effect of added chemicals on the heat resistance of Micrococcus pyogenes, var. aureus would be determined from survival curves plotted from experimental data. The capillary tube procedure, employing the apparatus in Section IVb is sufficiently accurate, but involves too much time to permit the rapid screening of many chemical substances in determinations of this type.

It was considered that a method which used larger amounts than 0.1 gram of homogenate would require less time. It was not necessary to measure exact thermal death rates, if relative lethal rates could be deter-

mined with the same conditions of heating. These conditions provided some latitude in the selection of a suitable procedure.

It appeared that a satisfactory method would be to use volumes of liquid substrate on the order of a few milliliters which could be heated in 20 x 150 mm bacteriological test tubes. This permitted the pipetting of samples, instead of the tedious filling and handling of the capillary tubes previously used. Volumes of this size are sufficiently small to minimize any great thermal lag in the heating or cooling of the sample. The selection of 140°F as a constant temperature for heating was based on the thermal death time results, which showed deviation from results reported in the literature for work carried out at temperatures above 140°F. This temperature also approximates the pasteurization range for milk, and has therefore, been used extensively in a number of previous heating studies with nonspore-forming bacteria.

Preliminary work was carried out on the conditions of heating and volumes of homogenate and chemical additive to find a specific set of conditions which could be held standard and would provide accurate information. It was found that 2.0 ml of homogenate and 0.5 ml of chemical were satisfactory volumes. Heating times of 0, 5, 10, 15, 20, 25, and 30 minute periods covered the bacterial survival range from 100 per cent to 0.00001 per cent.

Evaluation of resistance through this wide range is valuable because it spans the reduction in bacterial numbers from an initial population of 1×10^7 cells per gram to one cell per gram.

In addition, it is possible to observe all effects a material may offer, such as a decrease in the response time to heat, the direction and position of the survival curve, and any changes in slope of the curve. It was considered that this procedure, using survival curves, would give more information than the mere comparison of counts in similarly treated samples (test and control) at one or two points. It is possible to study the specific effect of an additive by this method by comparison of the survival curves.

As a basis for measuring effects, standard survival curves for both homogenates were established. An aliquot of 0.5 ml of sterile distilled water was added to each tube to simulate possible volume or dilution effects due to the addition of a similar aliquot of chemical. These control curves were standards for the purpose of comparison with curves influenced by chemical additives.

2. Equipment for Heating

The oil bath used in the thermal death time

apparatus was used for heating. It had temperature control within 0.1°C, a stirrer, and a volume of oil sufficient for consistent temperature maintenance. The capillary tube holder, cooling bath, and timing mechanism were not used.

A wire test tube rack with a capacity of 16 test tubes, and sufficient in size to fit within the oil bath, supported the tubes during heating.

Cooling was carried out in a portable bath, consisting of a water filled can within a quart-sized pan. The pan contained an ice-water mixture. The tubes were thus chilled immediately after heating, and held for plating.

3. Preparation of Homogenates

The preparation of homogenates, and all subsequent procedures, were identical to those described in Section IVb for ham.

The roast beef was from choice quality, top of the round. It was obtained weekly, and roasted in a gas oven at 375°F. Samples were obtained for use on the day of roasting. They were taken from below the surface, and the degree of roast could be described as "medium-rate". Fatty areas and connective tissue portions were trimmed prior to preparing the homogenate.

4. Preparation of Bacterial Suspension

The organism used was Micrococcus pyogenes, var. aureus, Strain 209. The culturing procedures and methods of preparing the inoculum were the same as those described previously in IVb.

The suspension was made by shaking packed cells with two successive 25 ml portions of sterile homogenate which were added to the centrifuge tube. The suspension was mixed at high speed in a Waring Blendor for one minute to produce proper texture and distribution. The suspension was removed directly from the Blendor cup to the sterile tubes with a sterile 10 ml graduated, wide-tip pipet. The tubes were stoppered with sterile cotton plugs.

5. Sample Preparation

Sterile test tubes were filled with 0.5 ml of the chemical solution being tested and plugged with sterile cotton. Six tubes were filled for each test material. Two other tubes serving as controls were filled with 0.5 ml sterile distilled water.

With a pipet, 2.0 ml portions of the suspension were added to each tube from the Blendor cup.

The tubes were swirled rigorously for mixing, special care being taken to avoid wetting the cotton plug, following which they were allowed to stand for 1 to 2 minutes to permit draining from the walls of the tube. The necks of the tubes were flamed to avoid the adherence of any suspension to the walls which might later contaminate the body of the suspension after heating, should the necks not be adequately heated by the oil. This was a precautionary step, which did not seem to affect the eventual counts. The necks of the tubes were marked with colored china marking pencil for identification.

The tubes were manually introduced and withdrawn from the oil bath. On removal from the bath, they were immediately wiped free from excess oil with a cloth, marked to show the duration of heating time, and then put into the chilled water bath, to be held until plating.

6. Preparation of Chemical Solutions

Solutions were prepared so that 0.5 ml added to 2.0 ml of homogenate would be of the desired experimental concentration. When two solutions were needed for a combination of additives, they were prepared so that 0.25 ml of each would produce the proper

concentration. This was done for the standardization of the method at a total volume of 2.5 ml of suspension.

Preparation of solutions was carried out daily with sterile distilled water. Solutions were diluted several times before the proper concentration for addition to the tubes was obtained. This was done to eliminate the possible weighing error when very small quantities are weighed.

All solutions used, except two, were of varying water solubilities, requiring, at the most, gentle warming with hot water and agitation. The two exceptions were sorbic acid and allylisothiocyanate. The latter, an oil, was emulsified with the required volume of dilution water in a Waring Blendor at high speed for two minutes. The emulsion was of sufficient stability to permit handling without separation. Because the solubility of sorbic acid increases with temperature, the solution and pipet were heated with free flowing steam, and pipetting was carried out quickly. To compensate for any changes in volume due to heating, two control tubes were filled with water at the same temperature.

The following is a list of the chemicals evaluated for both boiled ham and roast beef homogenates:

a. Roast Beef Homogenate

Acetic acid at pH 5.0
Sodium benzoate (0.10%)
Magnesium chloride (0.10%)
Calcium chloride (0.10%)
Sodium nitrite (200 ppm)
Hydroxylamine (100 and 200 ppm)
Hydroxylamine (200 ppm) and sodium nitrite (200 ppm)
Allylisothiocyanate (200 ppm)
l-Ascorbic acid (0.10%)
l-Ascorbic acid (0.10%) and hydroxylamine (200 ppm)
l-Ascorbic acid (0.10%) and sodium nitrite (200 ppm)
Sodium ascorbate (0.10%)
Sodium ascorbate (0.10%) and hydroxylamine (200 ppm)
Sodium ascorbate (0.10%) and sodium nitrite (200 ppm)
Sodium laurate (0.075 and 0.125%)
Sodium laurate (0.075%) and hydroxylamine (200 ppm)
Sodium laurate (0.075%) and sodium nitrite (200 ppm)
Sorbic acid (0.075 and 0.125%)
Sorbic acid (0.075%) and hydroxylamine (200 ppm)
Sorbic acid (0.075%) and sodium nitrite (200 ppm)
Calcium propionate (0.075 and 0.125%)
Calcium propionate (0.075%) and hydroxylamine (200 ppm)
Calcium propionate (0.075%) and sodium nitrite (200 ppm)
n-Propionic acid (0.075 and 0.125%)
n-Propionic acid (0.075%) and hydroxylamine (200 ppm)
n-Propionic acid (0.075%) and sodium nitrite (200 ppm)

b. Ham Homogenate

Acetic acid at pH 5.0, pH 5.5, and pH 6.0
Acetic acid at pH 5.0 and Hydroxylamine (200 ppm)
Magnesium chloride (0.10%)
Calcium chloride (0.10%)
Dimethyldihydroresorcinol (0.075%)
Sodium sulfite (0.075%)
Hydroxylamine (200 ppm)
Vitamin K_5 (10 and 100 ppm)
Allylisothiocyanate (200 ppm)
Allylisothiocyanate (200 ppm) and hydroxylamine (200 ppm)
Butylated hydroxytoluene (100 ppm)
Butylated hydroxytoluene (100 ppm) and hydroxylamine (200 ppm)
l-Ascorbic acid (0.10%)
l-Ascorbic acid (0.10%) and hydroxylamine (200 ppm)

Sodium ascorbate (0.10%)
Sodium ascorbate (0.10%) and hydroxylamine (200 ppm)
Sodium laurate (0.075 and 0.125%)
Sodium laurate (0.075%) and hydroxylamine (200 ppm)
Sorbic acid (0.075 and 0.125%)
Sorbic acid (0.075%) and hydroxylamine (200 ppm)
Calcium propionate (0.075 and 0.125%)
Calcium propionate (0.075 and 0.125%) and hydroxylamine (200 ppm)
n-Propionic acid (0.075 and 0.125%)
n-Propionic acid (0.075 and 0.125%) and hydroxylamine (200 ppm)

7. Preparation of Antibiotic Solutions

Antibiotic solutions were prepared in a similar manner to the chemical solutions so that 0.5 ml added to 2.0 ml of homogenate would produce the desired concentration, resulting in a standardized volume of 2.5 ml/tube.

Aureomycin (American Cyanamid) and Terramycin (Chas. Pfizer) were in a crystalline form as hydrochloride salts. Both were readily water soluble. Subtilin (U.S. Department of Agriculture) was of 70 per cent potency, and was dissolved in dilute hydrochloric acid (0.1%) at pH 1. Nisin (Aplin and Barrett Ltd.) had an activity of one million Reading Units per gram. Pure nisin has an activity of 40 million R.U./gram. It was dissolved like subtilin. The solutions were prepared in a concentration of 0.5 per cent of full potency antibiotic in 100 ml volumetric flasks.

During the period of use, Aureomycin and Terramycin stock solutions were prepared daily to eliminate any changes in potency with time. Subtilin and nisin, when present in high concentration, are reported to retain their potency in acid solution. (Hawley, 1957 and Dimick, et al, 1947). Because of this and the paucity of material, stock solutions were made, and used within four days. Solutions were kept refrigerated until use, and the flasks were wrapped with aluminum foil to eliminate effects due to light. Dilutions when needed were made from these stock solutions, which were allowed to come to room temperature before pipetting. These dilutions were discarded after use.

The concentrations of antibiotics in both homogenates were:

1 ppm
10 ppm
50 ppm

8. Incubation of Samples

Some of the chemical-containing ham substrates were incubated after being processed at 0 minutes (controls) and 30 minutes. This was carried out to find a chemical substance which would not only influence the rate of lethality during heating, but would also not

be destroyed during the heating and might then act after heating as a bacteriostatic agent to inhibit growth.

Sample tubes for incubation were processed concurrently with the samples of the same material for a given experimental run. Tubes were incubated for 24 and 48 hours at 37°C, and were then counted.

9. Counting of Treated Samples

After heat treatment, the tubes were held in the chilled water bath previously described. Sample counts were made from the tubes by removal of 1 ml portions to a dilution bottle containing 99 ml of sterile distilled water, providing a dilution of 1:100. To produce a dilution of 1:10, 0.1 ml of undiluted sample was placed directly into the Petri dishes. Before plating, the sample tubes were lightly agitated to resuspend the settled solids. Each dilution was plated in duplicate.

Counts were made in accordance with Standard Methods for the Examination of Dairy Products (1953) using Tryptone Glucose Extract Agar. Cultures were incubated at 37°C.

10. Treatment of Data

Duplicate counts were averaged for each tube. Counts were expressed on a one milliliter basis. Because the mean counts represented the extent of survival, the mean count of two unheated control tubes was considered to represent 100 per cent survival. Calculations of survival were made using this mean count as the divisor. The calculated "percent survival" values were plotted semilogarithmically for corresponding heating times.

All resulting curves were compared with the standard curves, derived from heating tubes at 140°F containing 0.5 ml of sterile distilled water. These curves were established by regression analysis (Appendix X-G).

B. RESULTS AND DISCUSSION

1. Chemicals

The survival curves at 140°F are shown in Figures 16 through 28 for ham, and Figures 29 through 39 for roast beef. Standard survival curves for ham and roast beef are shown in Figures 16 and 29, respectively. These control curves are based on survival with the addition of 0.5 ml sterile, distilled water to each tube.

In the evaluation of these curves, it is important to consider the basis upon which they were drawn. The purpose in drawing these curves was not primarily to establish exact rates, and changes in rates, of lethality for the individual additive. To do this it would have been necessary to have repeated each chemical series many times in order to obtain enough data to validate each segment of each curve statistically.

This work was set up as a rapid method to screen many chemicals. It was considered that the primary importance would lie in finding those materials that would have a marked effect on the lethal rate at 140°F. It was considered more nearly the purpose of this work

to determine the overall effects in the evaluation of chemical influence on bacterial destruction in meat during heating. The possible applications which might result from this work would be done with slices. Direct application of the values found for pure culture destruction in homogenate substrates would be impossible.

The value of this method lies in two areas. First, it is a screening method, which has been developed for evaluating lethal effects, providing information involving rates of destruction at a given temperature. This rate basis gives a better overall view than would result if either growth or no growth occurred in tubes after heating or if plate counts were compared at one condition of heating between homogenates with and without added chemicals.

Second, in a carefully controlled procedure, free of contaminating or resident organisms in an unvarying substrate, with consistent culture techniques, relating to age of cells, incubation temperatures and size of inoculum, it is possible to obtain an excellent indication of any alteration of heat resistance of microorganisms caused by the presence of an influencing substance.

The chemical substances used in this work are characterized by their potential suitability in foods. Many of the materials are in use. Because of this, concentrations of potential use or current allowance were

selected. Information was sought relative to direct application.

Because of the number of materials tested, the effect of each chemical agent on the destruction by heat of the test organism, Micrococcus pyogenes, var. aureus, is discussed separately. Comparison of curves is made relative to the standard curve for each substrate (Figure 16 for ham, Figure 29 for roast beef).

HAM HOMOGENATE

(1) The presence of 0.02% (200 ppm) of hydroxylamine produced a decrease in thermal resistance. The effect occurred after five minutes of heating, and increased with the time of heating, having an average difference of approximately one logarithmic cycle of survival.

(2) Heating in the presence of 200 ppm of allylisothiocyanate showed approximately the same order of decrease as hydroxylamine. The combination of 200 ppm of each chemical had no greater effect than either separately.

(3) The addition of acetic acid at pH of 6.0, 5.5, and 5.0 provided a rapid initial decline, compared with the control curve, but this higher lethal rate decreased with the time of heating. At pH 6.0,

only a slight variation from the control was seen, which was considered not to be significant. A greater initial lethality occurred at pH 5.5, reached a maximum of one log cycle of difference at 15 minutes, and then began a decrease until the 30 minutes heating period was reached, where the survival point was identical with the control. At pH 5.0, the curve was similar to that obtained at pH 5.5, but the overall effect was greater. At the 15 minute heating period, the difference was almost two log cycles; with a lethality slightly greater than that of the control at 30 minutes.

When 200 ppm of hydroxylamine were added at pH 5.0, the destructive effect of heating was slightly enhanced. At 30 minutes, there was a lethality increase of one log cycle beyond that obtained with the control.

The relatively low order of acid action might be supported by the many statements of the resistance of Micrococcus pyogenes, var. aureus cited in the Literature Survey. Apparently, a greater concentration of acid is required to produce a striking reduction in heat resistance than would be compatable with the flavor characteristics of meat.

HAM HOMOGENATE

(4) The addition of 100 ppm of butylated hydroxytoluene (B.H.T.) had no influence on the lethal effect of heat. The presence of 200 ppm of hydroxylamine combined with B.H.T. did have an effect, but this was of the order of the same concentration of hydroxylamine alone.

Interest in the possible bacterial effect of B.H.T. was based on its properties as an antioxident. If slices were ultimately to be held for some time in storage, ham, with its high fat concentration, might require the addition of an antioxident for the prevention of rancidity. As its presence might be required, it was considered that its bacterial properties should be evaluated.

(5) Heating in the presence of 10 and 100 ppm of Vitamin K_5 showed no greatly increased reduction in lethality. 10 ppm had no effect at all, while 100 ppm had only a slight effect. The high lethality reported in the Literature Survey of this material may have been affected by interferring substances within the ham substrate.

(6) The presence of 0.075% of sodium sulfite, 0.075% of dimethyldihydroresorcinol, and 0.10% of magnesium chloride (hydrated) had no influence upon the resistance to heat.

HAM HOMOGENATE

(7) Sodium laurate present in concentrations of 0.075% and 0.125% had a significant effect of approximately one log cycle throughout the entire heating period of 30 minutes. There was no difference between the two concentrations, except during the first five minutes of heating.

Sodium laurate is a soap. It is interesting to note that with both concentrations, there was a marked reduction in the lag period. At 0.075%, lethality began at 0.5 minutes and at 0.125%, enough of an initial effect occurred so that extrapolation of the curve to zero heating showed a reduction of 50 per cent. Most chemical additives did not show any alteration in the lag period before lethality occurred.

The addition of 200 ppm of hydroxylamine to 0.075% of laurate produced no added effect during the heating period.

(8) The presence of 0.075% and 0.125% of sorbic acid had no effect upon the lethal rate during heating. The addition of 200 ppm of hydroxylamine, together with 0.075% of sorbic acid, had a noticeable lethal effect, but one on the same order as that of hydroxylamine by itself.

HAM HOMOGENATE

(9) The presence of 0.10% of L-ascorbic acid and sodium ascorbate both had a noticeable effect upon the death rate. The difference between the two occurred maximally in the heating range of 15 to 20 minutes, where the ascorbic acid curve dipped substantially below that of the ascorbate. This difference was on the order of one log cycle, and decreased with the time of heating to 30 minutes, where the survival values were similar. At 30 minutes, there was a decrease of over one log cycle from the control curve.

The addition of 200 ppm of hydroxylamine to both ascorbic acid and its sodium salt did not change the extent of survival at 30 minutes, but had a slight effect during the heating period. With sodium ascorbate, the lethality was increased through 20 minutes, with a maximum effect at 15 minutes. There was a decrease in effect with addition of hydroxylamine to ascorbic acid, but this apparent effect was not great, and probably was due to experimental variation.

(10) The presence of 0.075% and 0.125% calcium propionate (Mycoban) had no effect upon the lethal rate. The two curves were almost identical with the standard curve.

HAM HOMOGENATE

Of great interest is the combination of 200 ppm of hydroxylamine and calcium propionate. Calcium propionate had no effect on the lethal rate by itself. The effect of the combination on the lethality was much greater than that of hydroxylamine as the sole additive. Similar curves were obtained for the combination of both concentrations of calcium propionate with hydroxylamine. The increased destruction rate began at five minutes with a difference of about a one-half log cycle, increased to almost 2 log cycles between 10 and 15 minutes, and continued parallel to the standard curve to 30 minutes. The magnitude of this rate of change in lethality showed a much greater increase than with any other chemical combination.

To study this phenomenon further, the same work was repeated using n-propionic acid. The presence of both 0.075% and 0.125% n-propionic acid showed approximately similar effects, with no great increase in the lethal rate. The higher concentration was slightly more effective. Both concentrations were not greatly different in effect from calcium propionate although there was a slight increase in lethality during heating.

HAM HOMOGENATE

The addition of 200 ppm of hydroxylamine to n-propionic acid produced an effect similar to that obtained with Mycoban and hydroxylamine. The increased lethality was very great. In all cases in which propionates and hydroxylamine were added, there was only a slight reduction in the time required for bacterial reduction to begin. The lethal rate continued to increase through the heating period.

ROAST BEEF HOMOGENATE

(1) The presence of both 100 and 200 ppm of hydroxylamine provided a noticeable reduction in resistance to heat. With the former concentration, maximum effectiveness occurred at 10 to 15 minutes of heating. Although the initial lethal rate was very high, it decreased after 15 minutes to the point where there was no difference from the control curve at 30 minutes.

Heating in the presence of 200 ppm produced a similar curve, but one indicating greater lethality. At a heating time of 10 minutes, there was almost two log cycles of difference from the control curve. This

difference gradually decreased to almost one log cycle at 30 minutes.

(2) Sodium nitrite in a concentration of 200 ppm had a high initial bactericidal effect during heating, through the first 15 minutes, with a maximum difference of almost one log cycle. With continued heating, the lethal effect decreased until there was little difference at 30 minutes. The destruction curve obtained with this concentration of nitrite was quite similar to that of 100 ppm of hydroxylamine.

The combination of 200 ppm of hydroxylamine with 200 ppm of sodium nitrite caused a decrease in the rate of survival equivalent to almost one log cycle greater than that of the nitrite alone. This combination had a very significant bactericidal effect, but it was not much greater than that obtained by the addition of 200 ppm of hydroxylamine alone.

(3) The addition of 0.10% of calcium chloride had no significant effect upon the death rate. The same results were obtained with magnesium chloride. The calcium salt had an initially high rate change, but it was not significant.

(4) The presence of 200 ppm of allylisothiocyanate had a significant effect in decreasing the thermal re-

sistance. The decrease was quite similar to that obtained with 200 ppm of hydroxylamine.

(5) The addition of acetic acid to provide a pH of 5.0 had a substantial effect on lethality associated with heat. The lag time was reduced, and the lethality during the first 15 minutes of heating was great. At 15 minutes, there was about two log cycles of survival difference between the standard curve and that influenced by acetic acid. After 15 minutes, the rate of lethality was decreased, and at 30 minutes, there was a difference of one log cycle.

(6) Sodium benzoate in a concentration of 0.10% had a slight effect on the overall destruction rate during heating, but this effect was not important.

(7) Concentrations of 0.075% and 0.125% of sodium laurate had an initially high lethal effect, which decreased with increased heating time. At 15 minutes, the difference in lethality in the presence of 0.075% of laurate compared to the control curve was greater than one log cycle of survival, but at 30 minutes, there was no difference with the standard curve. The curve influenced by 0.125% paralleled that of the lower concentration, but gen-

erally indicated a greater effect. In this case, the difference in survival at 15 minutes was greater than one log cycle, compared to the control curve, and was less than one at 30 minutes.

The combination of 200 ppm of hydroxylamine and 0.075% sodium laurate increased the lethal rate quite significantly. The initial rate was high through the first 10 minutes of heating, and was roughly parallel to the standard curve to 30 minutes. At 10 minutes, the variation from the control was two log cycles of survival, and more than one at 30 minutes.

The combination of 200 ppm of sodium nitrite with 0.075% sodium laurate had an effect similar to that of the same concentration of hydroxylamine alone.

(8) The presence of 0.075% and 0.125% sorbic acid only slightly affected the death rate associated with heating, especially the lower concentration. The curve obtained with 0.125% was parallel to that of 0.075%, but indicated a greater lethal effect on the order of one half log cycle. The presence of 0.125% sorbic acid had a fairly substantial effect at 15 minutes, shown by a difference of more than one log cycle, but this decreased with heating time to less than one half log cycle at 30 minutes.

ROAST BEEF HOMOGENATE

The lethal rate became significant with the addition of either 200 ppm of hydroxylamine or sodium nitrite. There was no essential difference between the two additives. The initial effect was very great, but changed at 10 to 15 minutes heating to a lower rate. At the maximum effect, the difference was approximately two log cycles from the control. The overall effect was quite similar to the effects of 200 ppm of hydroxylamine alone.

(9) Concentrations of 0.10% L-ascorbic acid and sodium ascorbate both showed a great increase in the thermal lethal rate. The ascorbate seemed to have a slightly greater influence upon the lethality. Both curves are of the same order as that obtained solely with 200 ppm of hydroxylamine.

The addition of 200 ppm of sodium nitrite or 200 ppm of hydroxylamine to 0.1% ascorbic acid increased the rate of thermal destruction. Hydroxylamine was more effective than nitrite by approximately one log cycle. Comparison with the standard curve showed a gradual increase in the lethal rate with increased time for the nitrite and ascorbic acid combination to 30 minutes, where the difference was two log cycles of

survival compared with the control curve. Combined with hydroxylamine, the acid showed a more rapid increase in lethal effects with increased heating time. At the 30 minute heating time the difference was over two log cycles relative to the control curve.

Both 200 ppm of sodium nitrite and hydroxylamine affected the lethal rate when combined with sodium ascorbate. The three curves obtained from ascorbate alone, ascorbate and nitrite, and ascorbate and hydroxylamine had the same lethal rates during heating to 10 minutes, after which, the effect of hydroxylamine was slightly greater than nitrite, which in turn, was greater than the ascorbate alone. The difference between these curves amounted to about one log cycle at 30 minutes.

(10) The presence of 0.075% of calcium propionate had a slight, but non-significant, effect upon the lethal rate. The concentration of 0.125% calcium propionate had approximately twice the effect, which was not very great.

Both 200 ppm of hydroxylamine and sodium nitrite when combined with 0.075% propionate had similar lethal effects, which were at a maximum at 15 and 20 minute heating times, where the difference from the control

curve was on the order of over two log cycles of survival. At 25 and 30 minutes, the effect decreased until there was approximately slightly more than one log cycle of difference with that of the control.

As with ham, the effects were much greater than those obtained with only hydroxylamine in the medium. Work was continued with the acid of this salt.

The presence of both 0.075% and 0.125% n-propionic acid had a noticeable influence on the thermal death rate, the latter having a slightly greater effect. The nature of the curve influenced by 0.075% was remarkably similar to that obtained with 200 ppm of hydroxylamine.

There was no change in the curve based on the presence of 0.075% of the acid when 200 ppm of either sodium nitrite or hydroxylamine were added.

A characteristic survival curve, resulting from the addition of chemical substances, has been seen through the heating time of 0 to 30 minutes at 140°F. The nature of this curve may be explained in several ways, assuming that the addition of a chemical material results in some lethality of Micrococcus pyogenes, var. aureus beyond that associated with heating. As shown

in the plotted results, the curved feature may occur even when the additional lethality is not very great. The control curve, with no additive is linear.

With some additives, the increased lethal effect is lost during heating. Two general types of chemical reactions may be responsible for this loss. Direct heat damage may occur, resulting in an ineffective product, which is not lethal to the bacterial cells. It is also possible that the chemical might be inactivated by chemical combination with the substrate. Thus, during the early stages of heating, the rate of lethality was increased beyond that associated with heat due to the influence of the chemical. During continued heating, this loss in activity increased as the inactivation reactions proceeded, and with it, the effect on the rate of bacterial destruction is decreased. This decrease of effect was responsible for the change in slope, until, in the later stages, little difference may be seen in survival from the control curve. The rate of chemical inactivation would determine the influence on the lethal rate of heating.

It is also possible that the initial rapid increase in lethality during heating decreases with time because the organisms affected are those that would have been destroyed anyway in the later heating phase. This could have caused an accelerated initial rate, which decreased with time.

Although statistically there is no significant difference between the standard curves obtained for ham and roast beef, there appears generally an increased response in the roast beef substrate with the addition of chemicals. This would seem to be contrary to the results obtained with meat slices, but may not necessarily be so. First, with slices the rate of destruction of Micrococcus pyogenes, var. aureus in ham during heating, was not much greater than that obtained with roast beef, although the difference was consistent. Also the physical characteristics of the media are different.

Ham slices present a totally different substrate than do diluted ham solids in homogenate form. In homogenates, it is possible that the difference in fat contents may have some influence. As cited in the Literature Survey, fat may exhibit a protective effect for organisms subjected to heating. Fat is in an emulsified form in homogenates, and in this state, may be protective. Winton and Winton (1938) showed that top of the round beef has half the average fat content of boiled ham. The average values were 11 per cent and 23 per cent, respectively. This fat ratio would still hold for the 2:1 dilution of the homogenate.

The nitrite content of the ham was diluted in the homogenate, possibly to a level where its influence

upon the lethality associated with heat would be brought to negligible levels. It was speculated that the presence of nitrite in the slice might contribute to the lethality. Eliminating nitrite as a factor on this basis, the differences in survival during heating between ham and roast beef homogenates containing chemicals might well rest with the respective fat contents.

The most effective chemicals in ham were hydroxylamine, allylisothiocyanate, sodium ascorbate and ascorbic acid singly and each in combination with hydroxylamine, and the combinations of both calcium propionate and propionic acid with hydroxylamine. In general, the addition of hydroxylamine produced an increased rate of lethality during heating. Propionate and ascorbate combinations, however, were the most active. With propionate alone, little effect was seen, but the addition of hydroxylamine produced an extremely high order of lethality. The addition of ascorbate alone showed markedly high lethality during heating. The addition of hydroxylamine produced an additional increase in microbial lethality due to heat.

With roast beef, the same chemicals and combinations were effective. In addition, 0.125% sorbic acid and 0.075% sorbic acid combined with either hydroxylamine or nitrite, 0.125% sodium laurate and

the combination of 0.075% laurate and hydroxylamine, and acetic acid at pH 5.0 were highly effective in accelerating lethality during heating.

The combination of 200 ppm of hydroxylamine with many of the substances produced an effect not in excess of that observed when the same concentration of hydroxylamine was used alone.

Hydroxylamine at a concentration of 200 ppm was more effective than sodium nitrite at the same concentration, both alone or in combination with other agents. A concentration of 100 ppm of hydroxylamine did not have a significant effect.

The contribution of the ascorbate radical to the lethal rate associated with the heat destruction of microorganisms is valuable. Because of the chemical protection afforded by ascorbate to meats, its use would be indicated even if it had no bactericidal properties. Its lethal effect is enhanced in the presence of hydroxylamine or nitrite, which makes its use in ham, with normal nitrite levels, more valuable.

Some indication was sought concerning any further bacterial effects which these materials might have after heating. Preliminary work showed that the chemicals, used in the range of concentrations employed, had no bacteriostatic or bactericidal effects without heating. If any

potential effect existed, it might well have been obstructed by the large numbers of bacteria involved. After heating, however, the numbers are considerably lower. It was considered that the value of an additive would be substantially heightened if it had the further property of inhibiting growth after heating.

To discover the presence of such a continuing effect, work was carried out with ham. This substrate was selected because it seemed to offer more resistance to lethal effects due to chemicals during heating.

The chemicals tested, and the mean counts during incubation, are shown in Table 1.

The only chemical found to have a bacteriostatic influence after heating was 200 ppm of hydroxylamine. With some materials, growth did not occur as rapidly as it did in the control tubes, but none of these was of the magnitude of the hydroxylamine effect. At a concentration of 100 ppm, hydroxylamine was not inhibitive, indicating that the critical concentration lay between 100 and 200 ppm.

Because hydroxylamine had a duality of effects, that of enhanced bacterial reduction with heat, followed by continued bacteriostasis, some thought was given to its use in foods. There is now no regulatory provision for the addition of hydroxylamine to foods. There has

been little interest within the food industry for its inclusion in foods, and there is an absence of reports in the literature on the subject of dietary toxicology and pharmacology.

The Handbook of Toxicology, edited by Spector (1956), lists the following toxicological data. For interest, the data for sodium nitrite is also included:

Substance	Test Animal	Administration	Lethality
Hydroxylamine*	Rabbit	sc	LD 25 mg/kg
	Dog	or	LD 200 - 300
	Dog	iv	LD 60
Hydroxylamine·HCL	Mouse (f)	-	LD_{50} 408 mg/kg
	Mouse (m)	-	LD_{50} 417
Sodium Nitrite	Frog	sc	LD 1000 mg/kg
	Rat	sc	MLD 10 - 20
	Rabbit	sc	MLD 60
	Rabbit	sc	LD_{100} 170
	Rabbit	iv	MLD 80 - 90
	Cat	sc	LD_{100} 35
	Dog	or	MLD 330
	Dog	sc	MLD 50 - 70

* Results of work carried out in 1888 and 1883.

This information is not sufficiently conclusive to enable a definite statement about the toxicity of hydroxylamine to be made. However, it would seem that hydroxylamine is slightly higher in toxicity than sodium nitrite, which despite its inclusion in cured meats, is a relatively toxic material.

Lehman (1957) stated that the U.S. Food and Drug Administration has no data on the chronic toxicity of hydroxylamine, and would consider it a toxic substance unless satisfactory evidence to the contrary were presented.

On the other hand, nitrites are permitted in cured meat products, and it may be possible to show that projected levels of hydroxylamine use may be no more deleterious to the human than the dietary nitrite the average human now consumes in meat products. Proof of this would have to be borne by those considering its use.

It would seem that the addition of some commonly used chemical materials to foods to be pasteurized may have distinct bacteriological advantages which, in concentrations normally used, act synergisticly with heat to increase the destruction of Micrococcus pyogenes, var. aureus.

TABLE 1

Growth in Tubes After Heating for 30 Minutes at 140°F

The Influence of Chemical Additives to Inhibit Growth.

Incubation at 37°C.

Sample	Mean T.G.E. Plate Count at 37°C. Time of Incubation (hours)		
(1) Added Chemical			
(2) Heating Time (min. at 140°F)	0	24	48
Control (0%)			
0	160×10^6	65×10^7	280×10^6
30	80×10^3	55×10^7	35×10^7
Vitamin K_5 (100 ppm)			
0	--	50×10^7	155×10^6
30	50×10^3	200×10^6	135×10^6
Calcium Propionate (0.125%)			
0	--	295×10^6	210×10^6
30	130×10^2	30×10^7	150×10^6
Sodium Laurate (0.125%)			
0	--	115×10^6	140×10^6
30	250×10^1	190×10^6	65×10^7
l-Ascorbic Acid (0.1%)			
0	--	80×10^7	280×10^6
30	45×10^1	35×10^7	250×10^6
Sorbic Acid (0.125%)			
0	--	50×10^7	35×10^7
30	155×10^1	230×10^6	40×10^7

(continued)

TABLE 1 (continued)

Sample	Mean T.G.E. Plate Count at 37°C. Time of Incubation (hours)		
Acetic Acid (pH 5.0)			
0	--	50×10^7	30×10^7
30	35×10^3	170×10^6	390×10^6
Allylisothiocyanate (200 ppm)			
0	--	40×10^7	150×10^6
30	165×10^1	205×10^7	115×10^6
Hydroxylamine (100 ppm)			
0	--	65×10^7	165×10^6
30	150×10^2	45×10^6	100×10^6
Hydroxylamine (200 ppm)*			
0	--	50×10^7	225×10^6
30	40×10^2	45×10^2	60×10^2

* Mean plate count of duplicate tests.

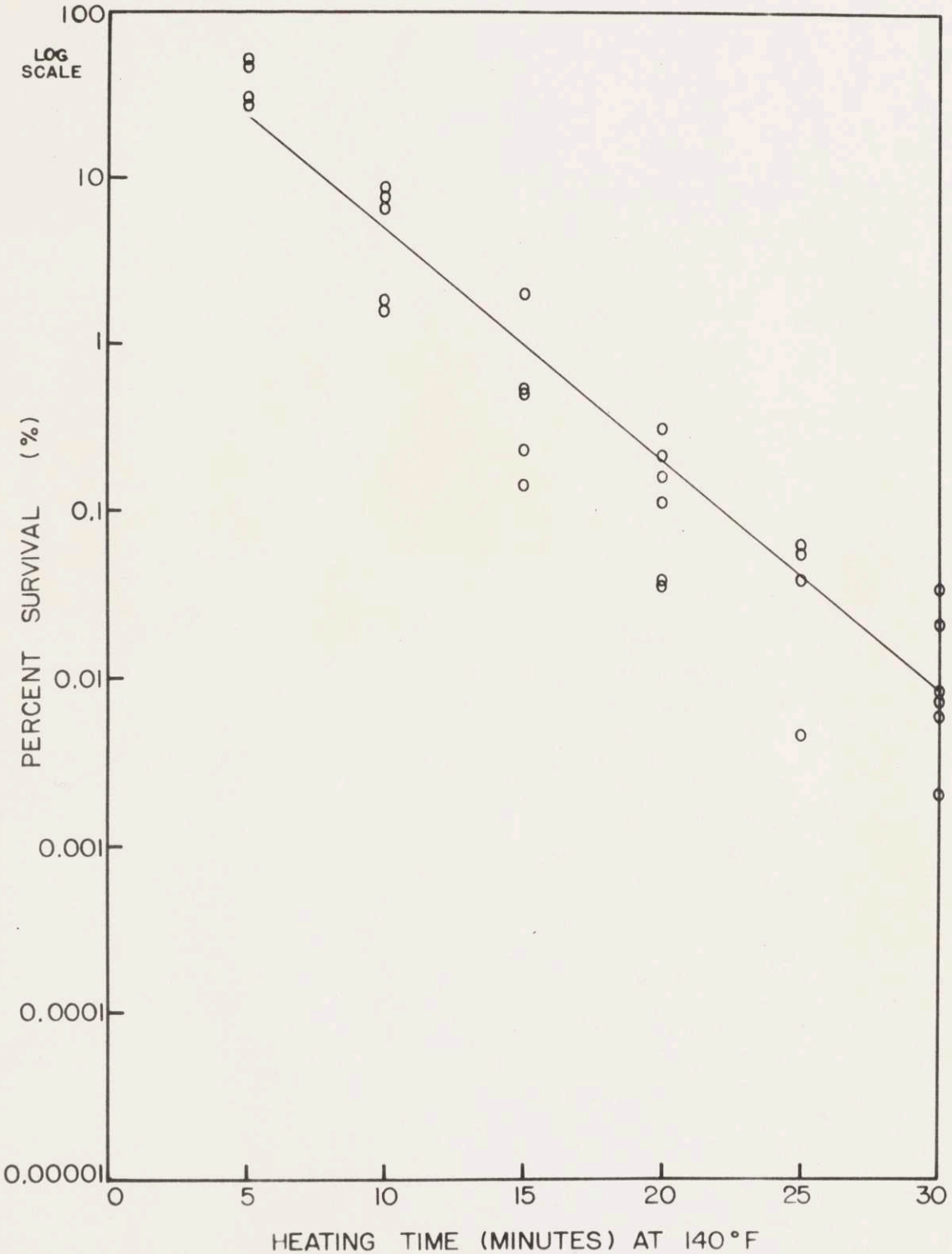

Figure 16. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Control Curve for Effects of Additives.

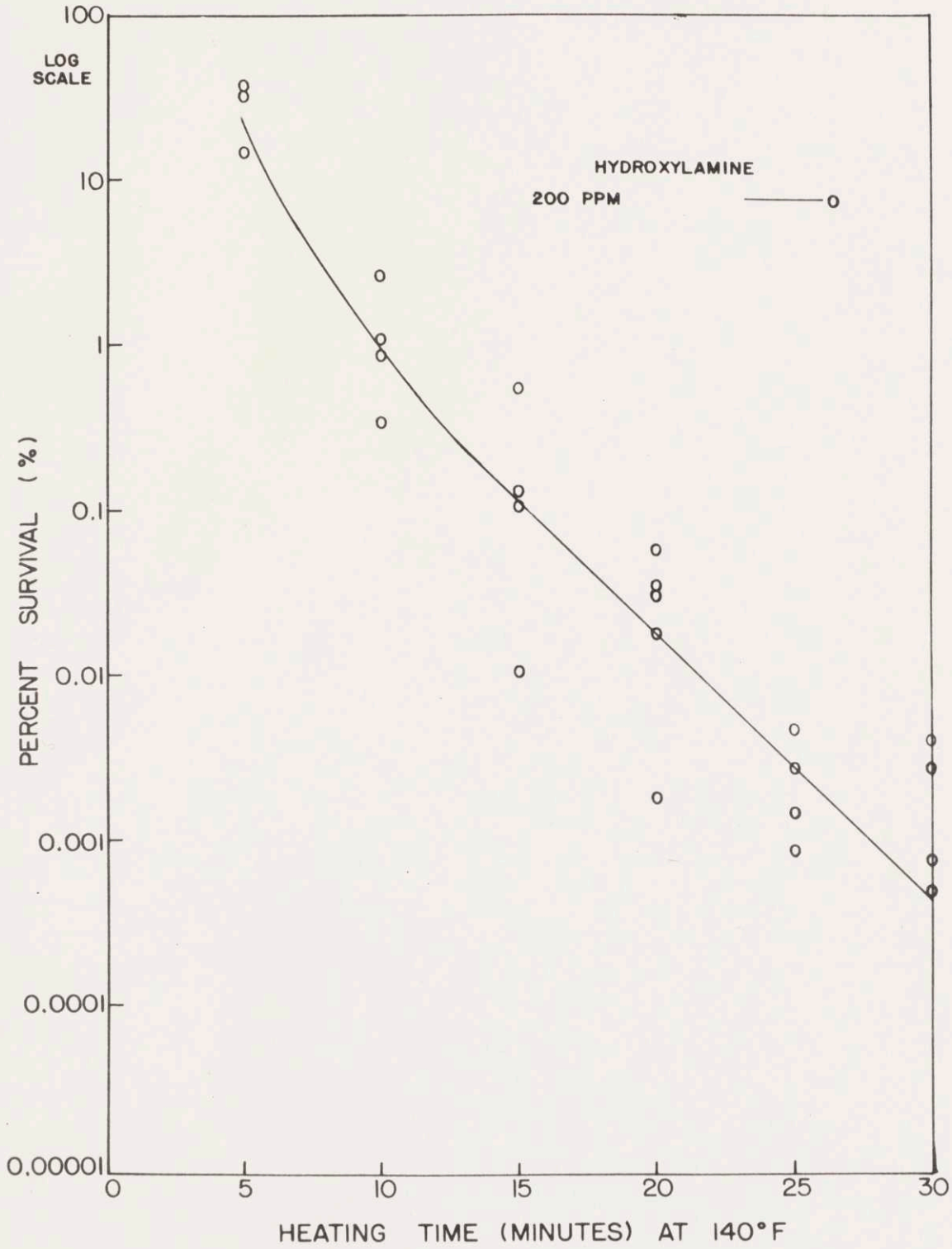

Figure 17. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

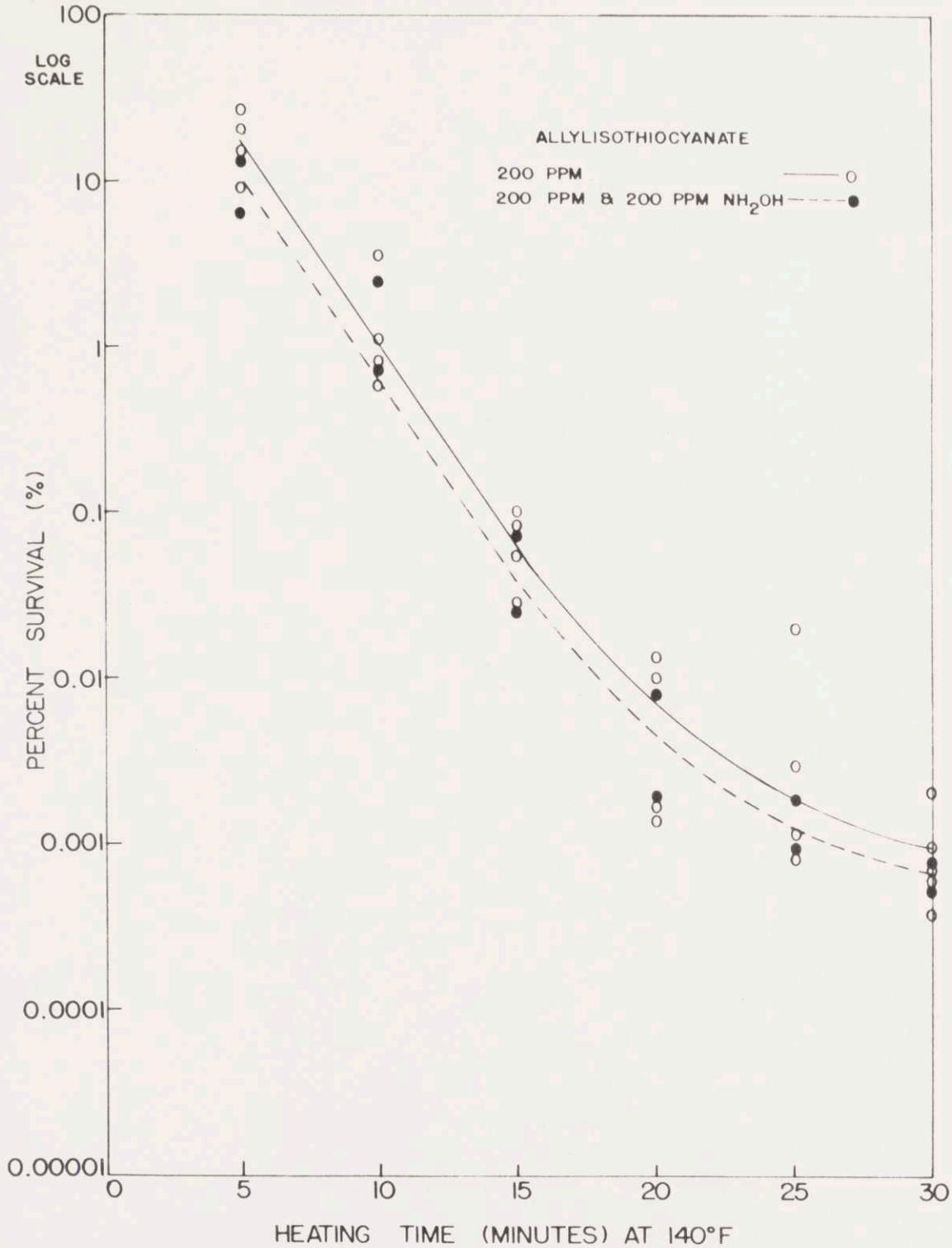

Figure 18. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

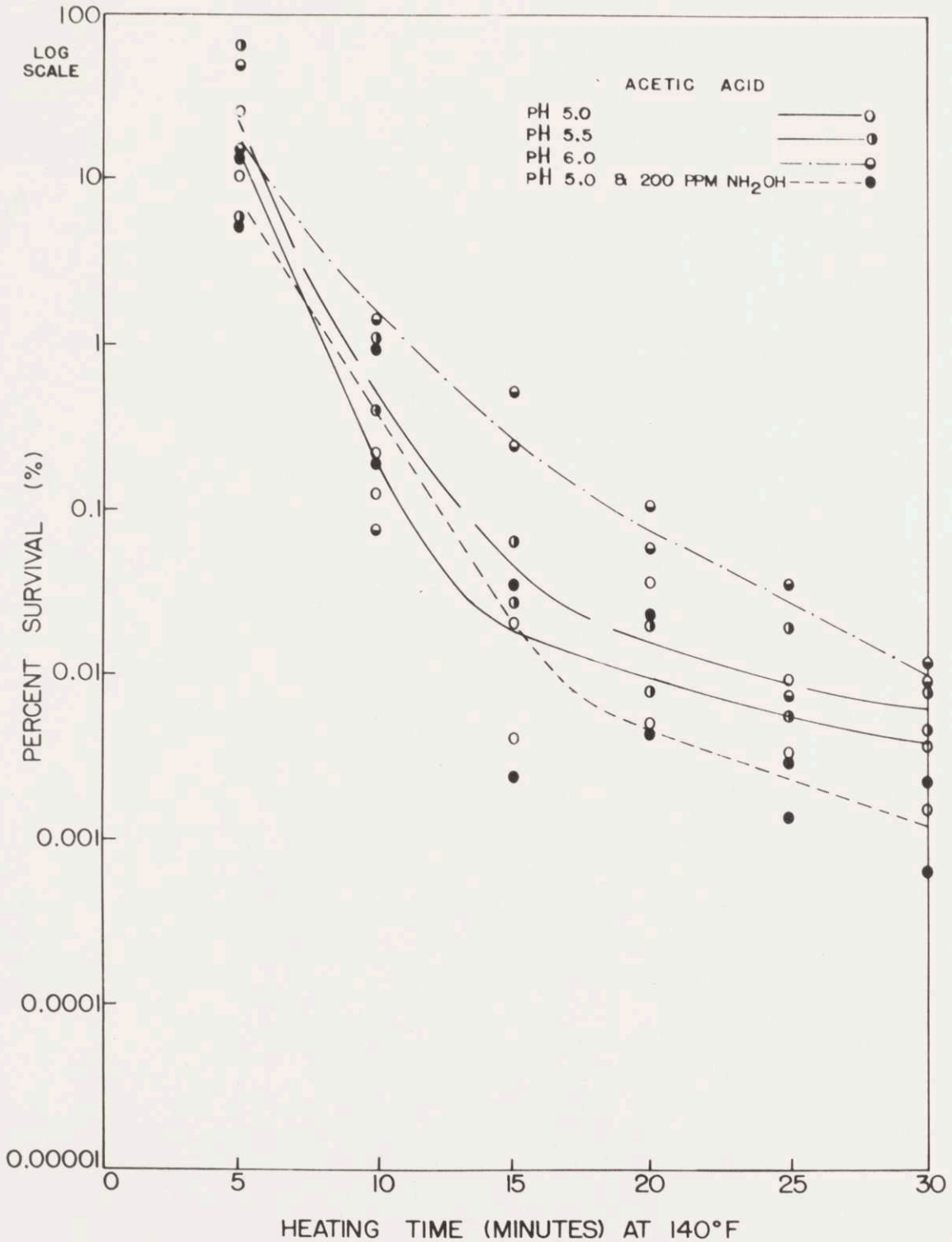

Figure 19. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

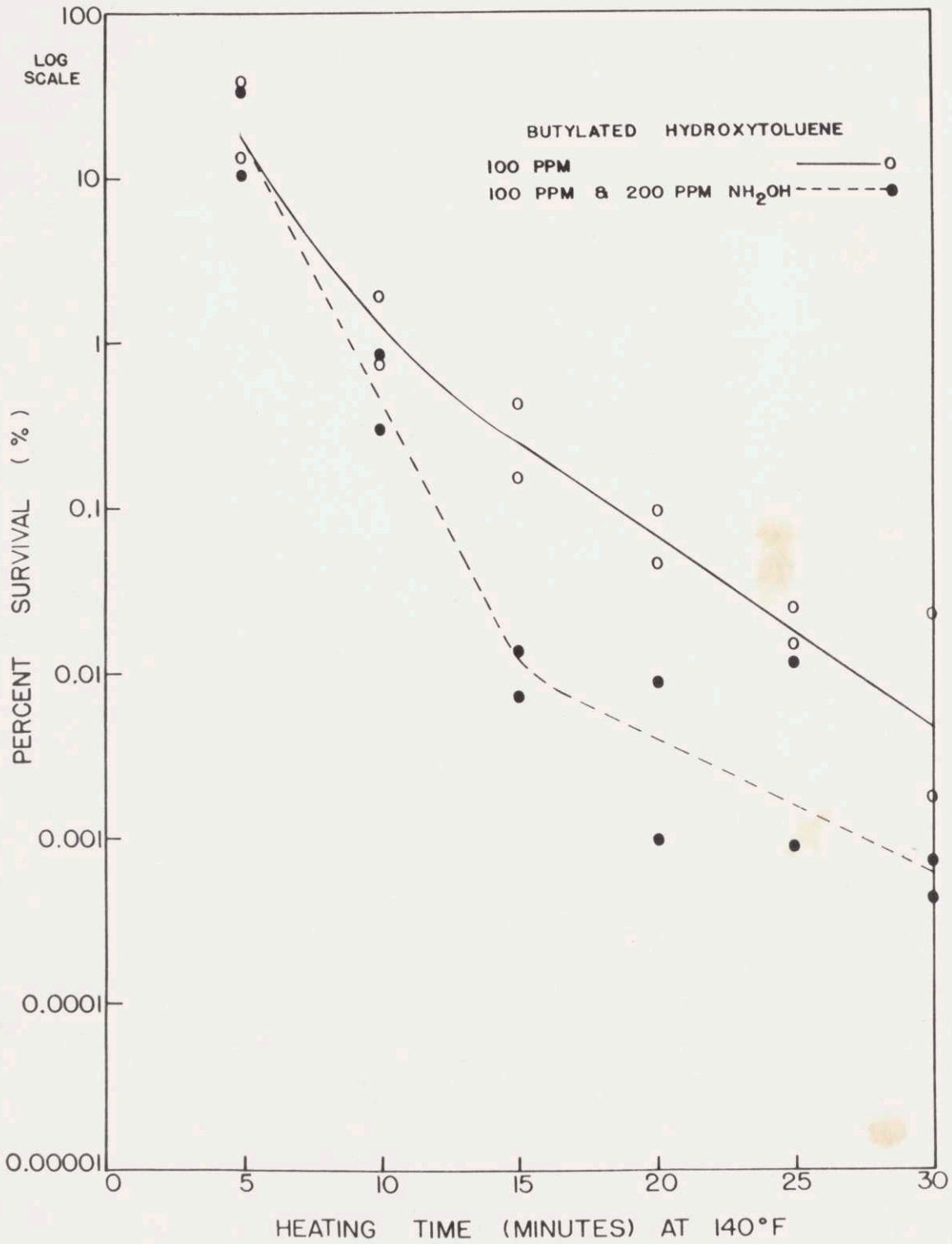

Figure 20. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

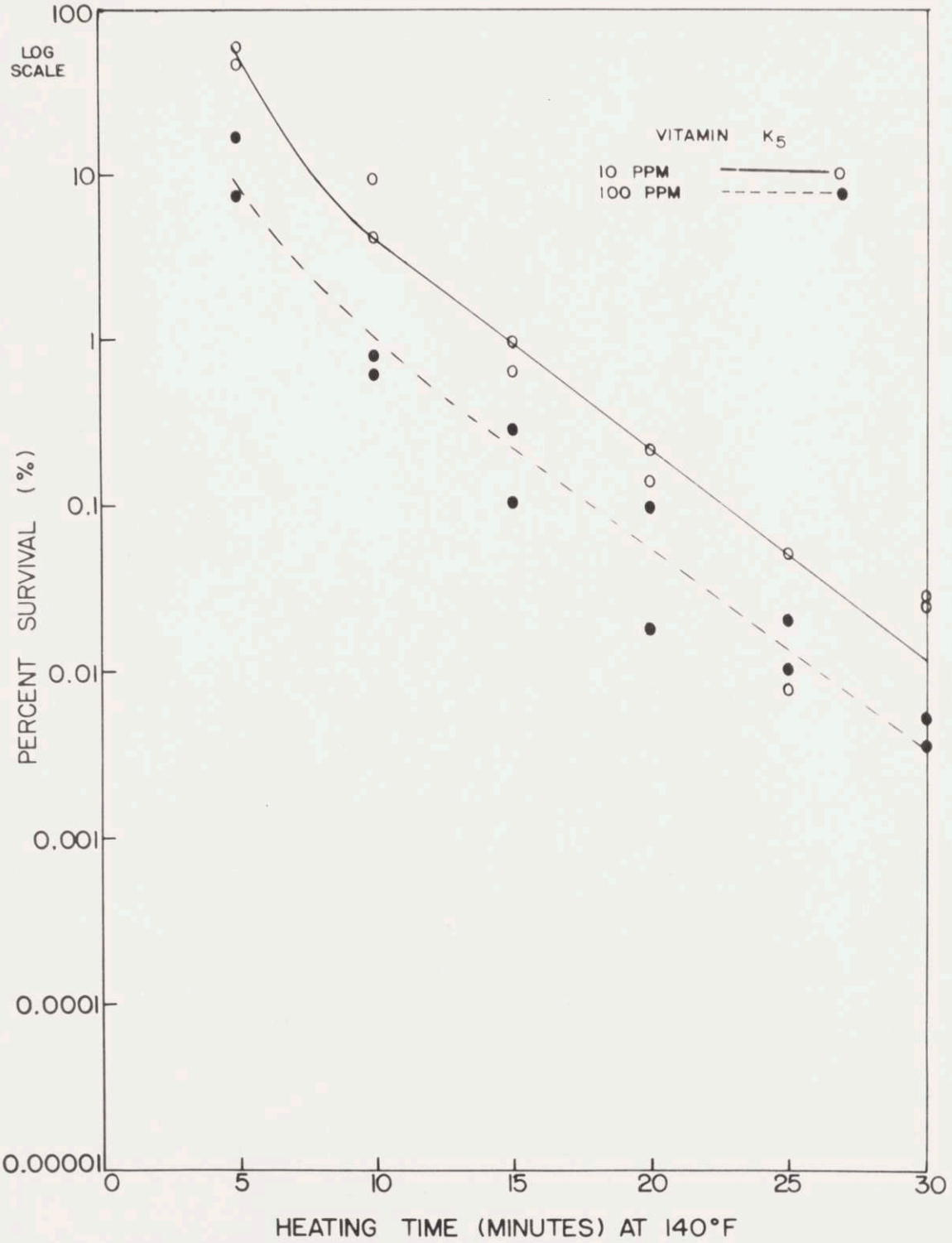

Figure 21. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

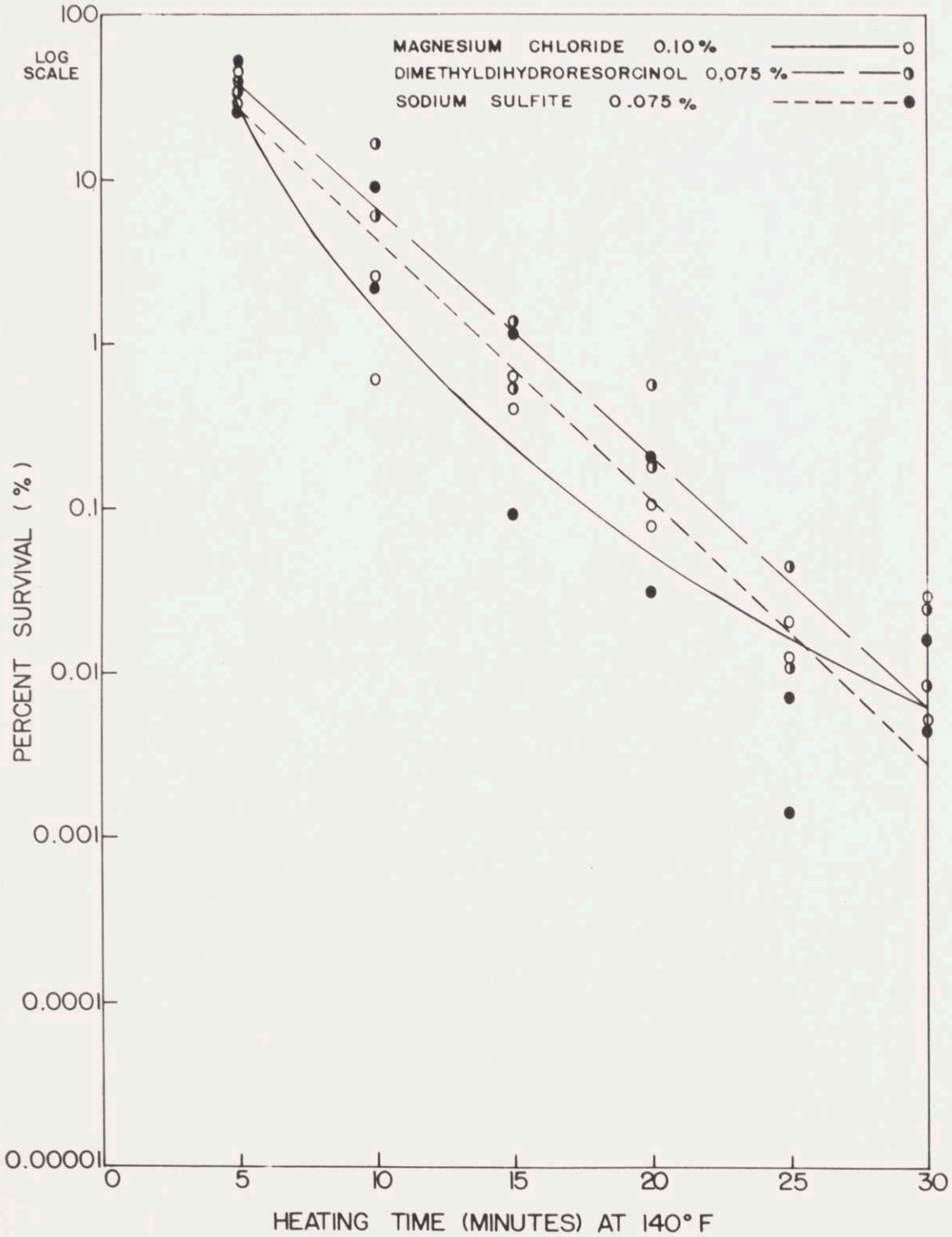

Figure 22. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

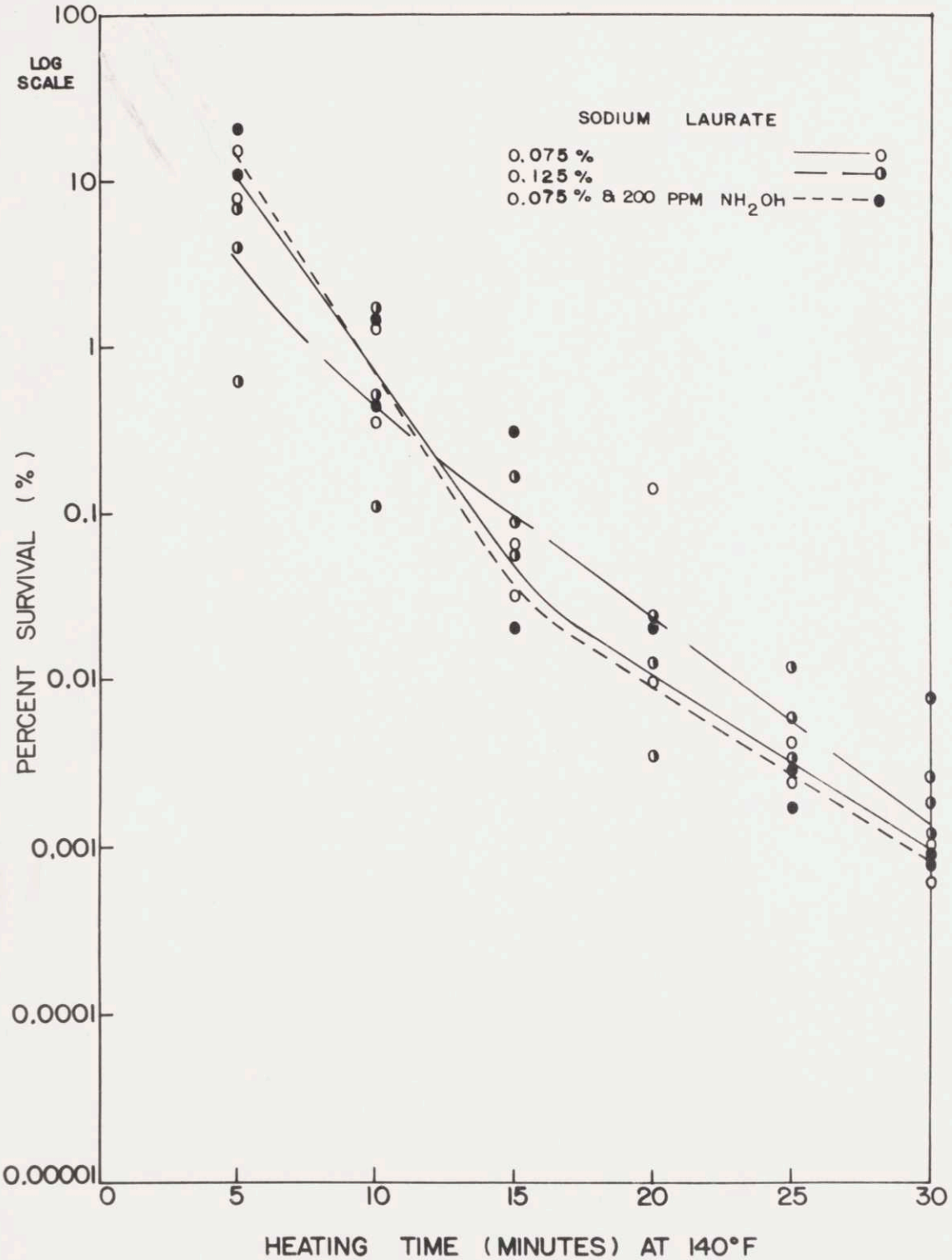

Figure 23. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

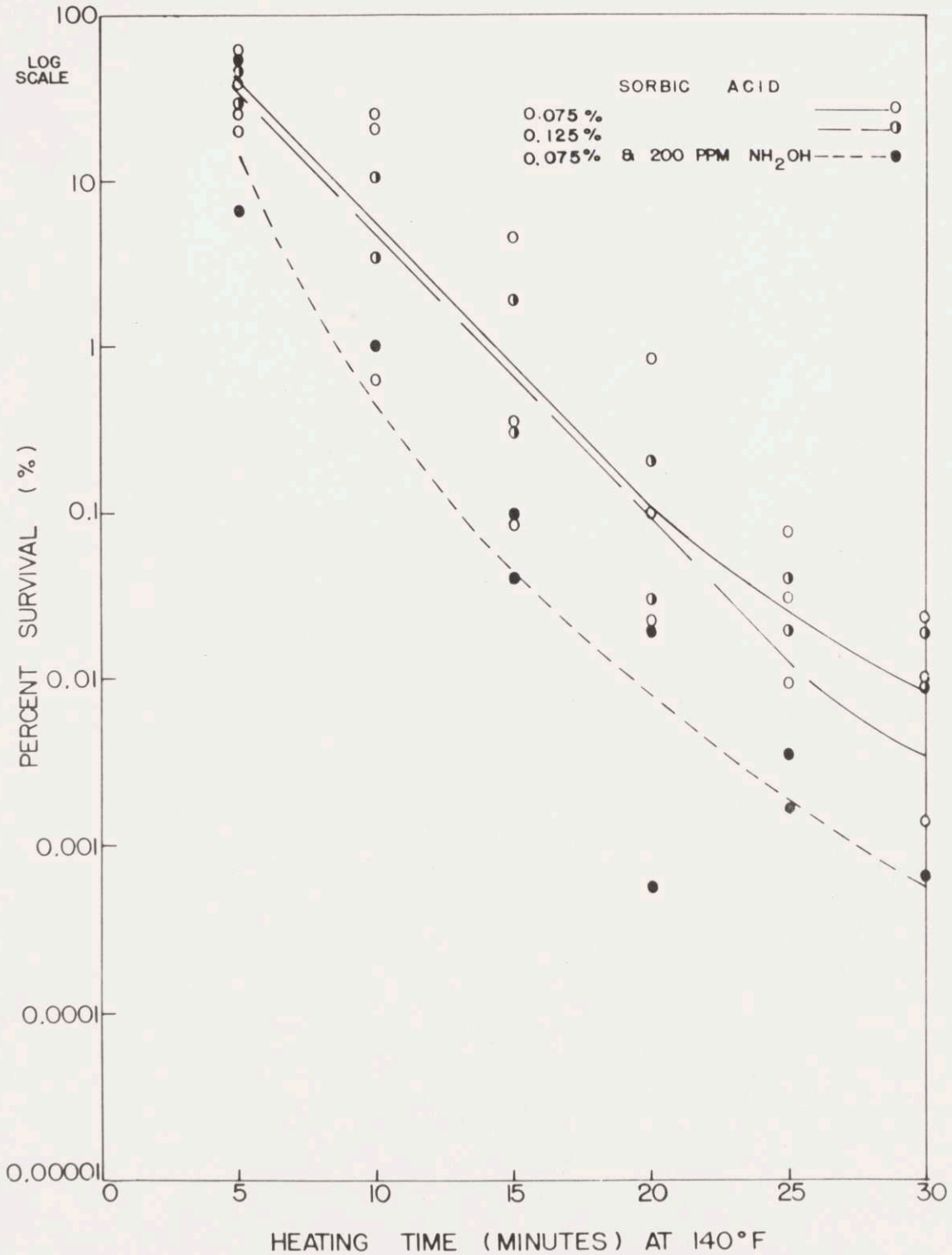

Figure 24. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

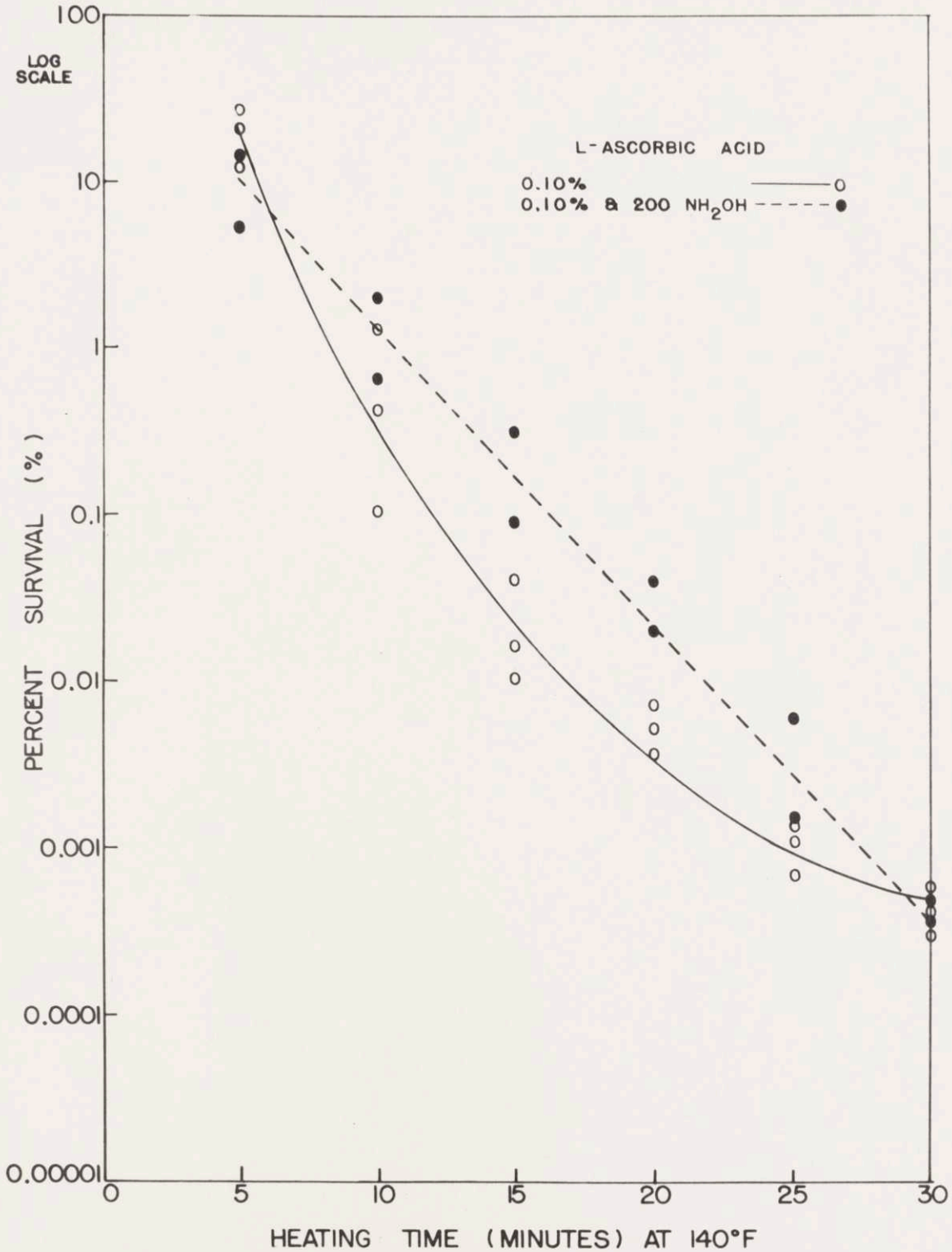

Figure 25. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

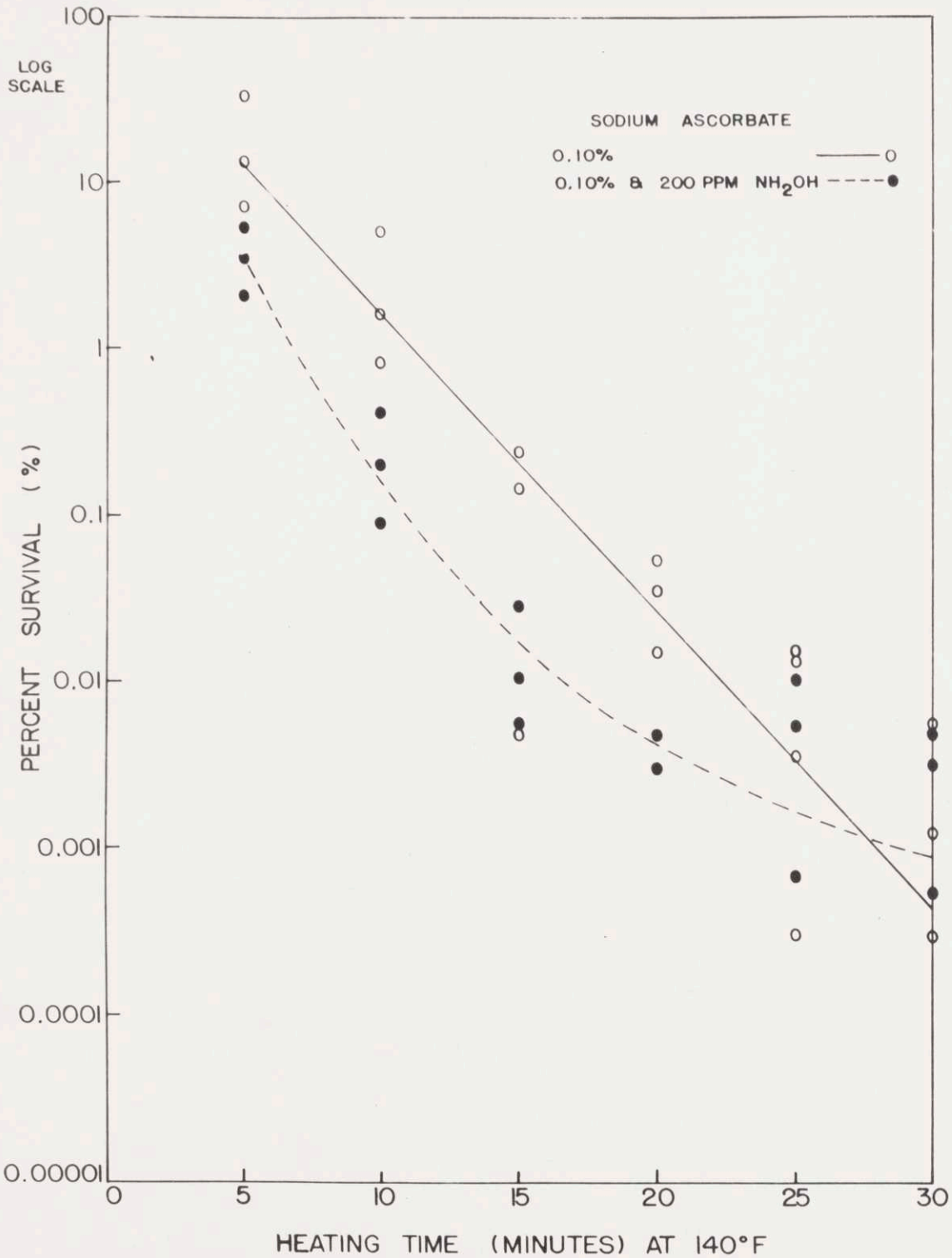

Figure 26. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

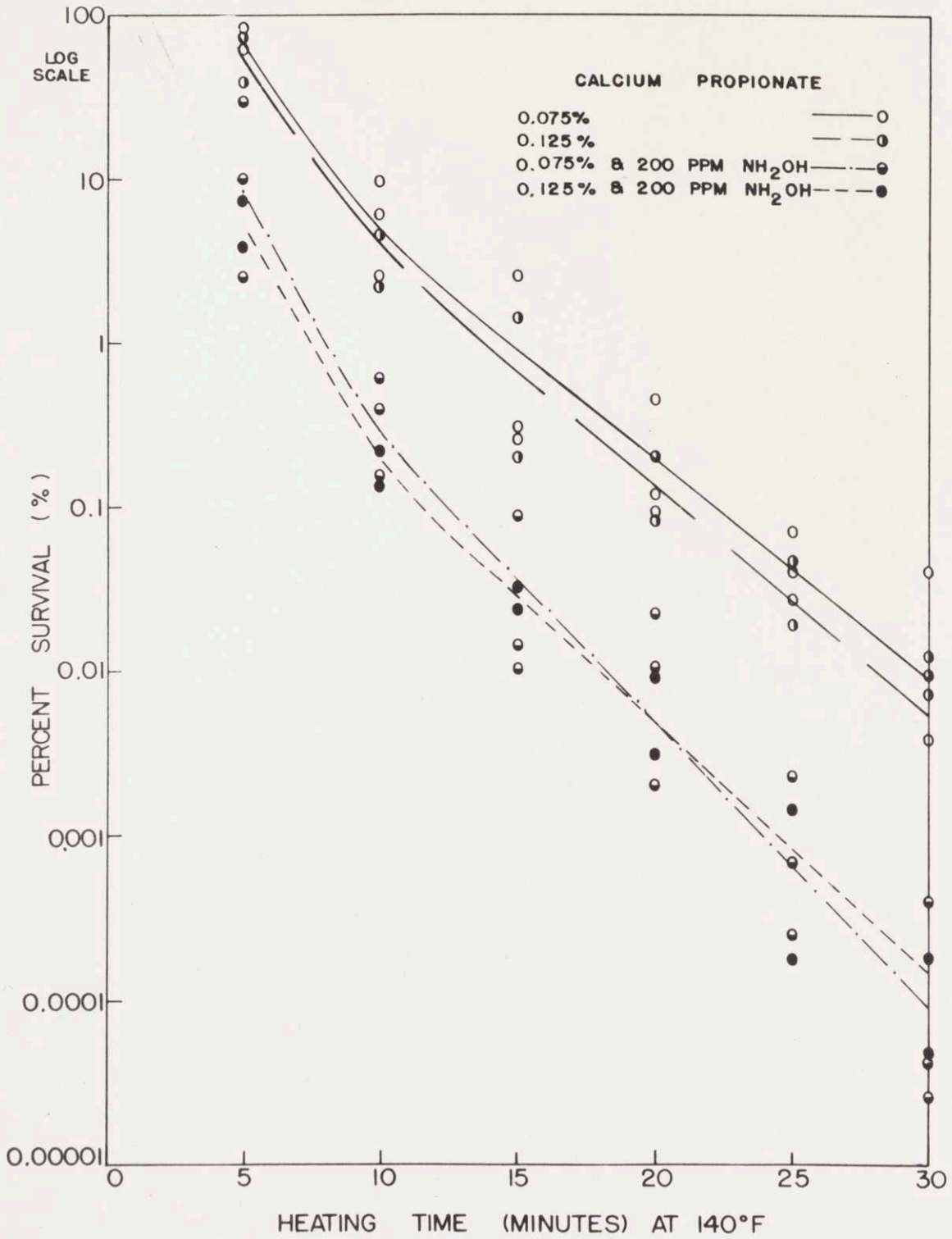

Figure 27. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

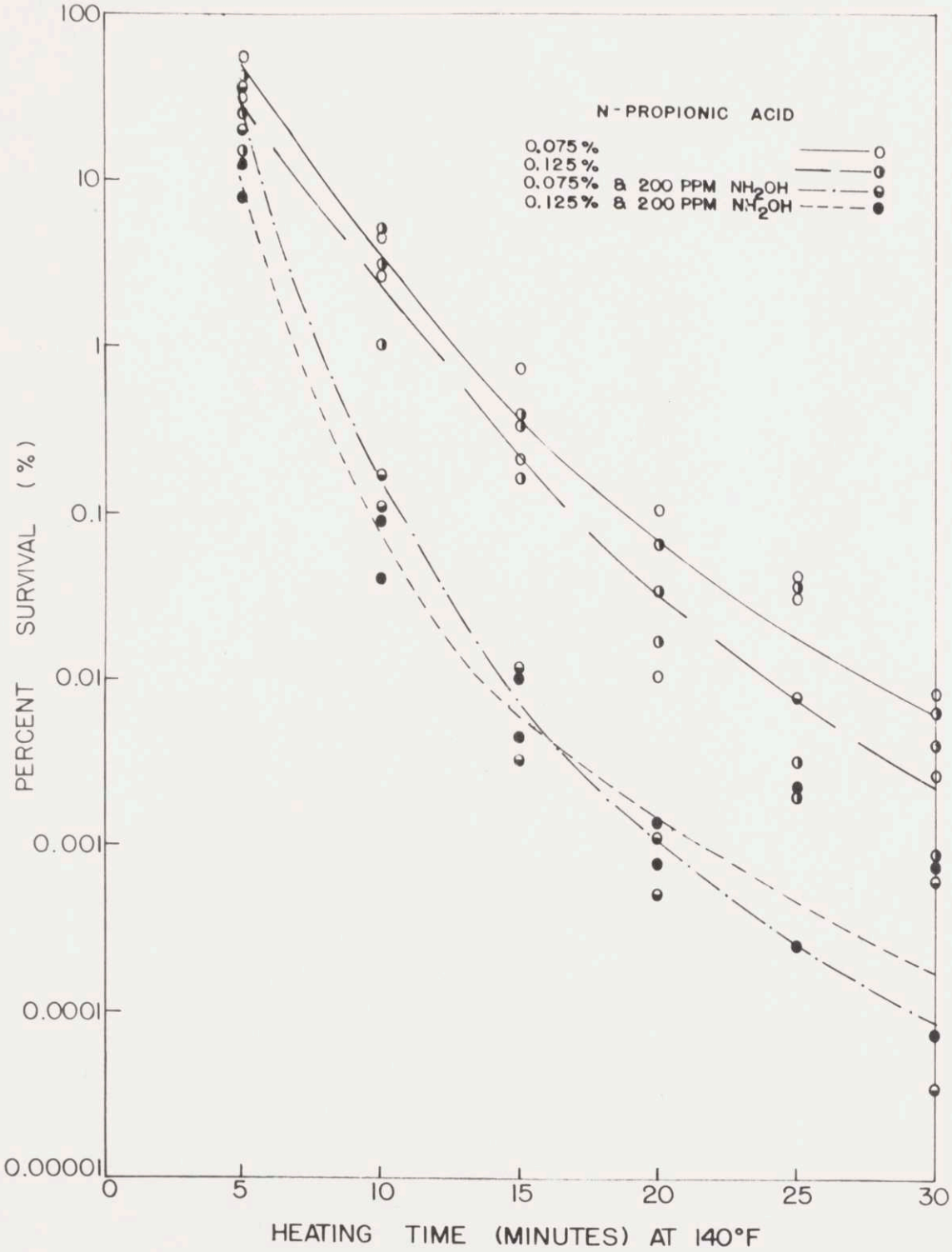

Figure 28. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. Chemical Present in Homogenate.

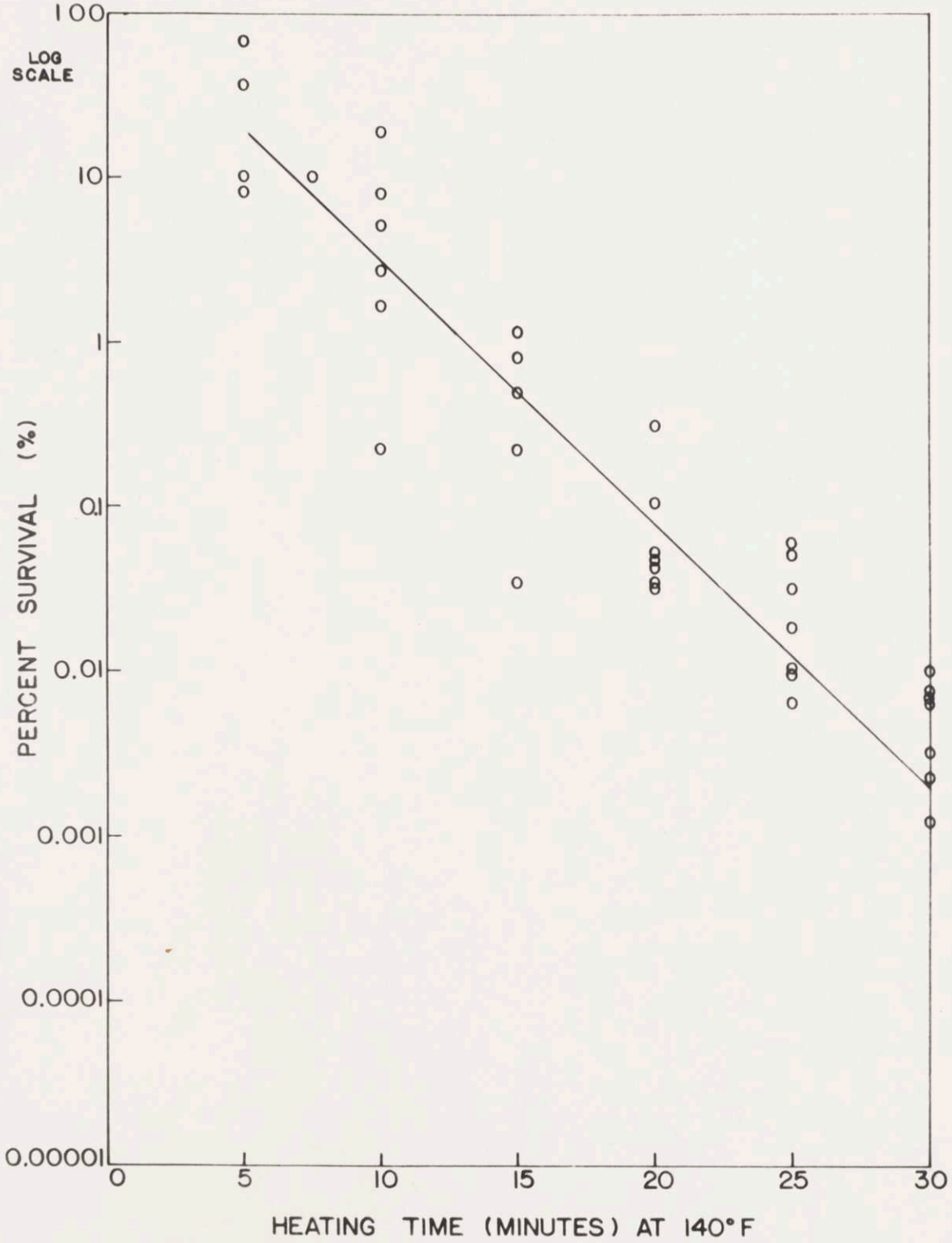

Figure 29. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Control Curve for Effects of Additives.

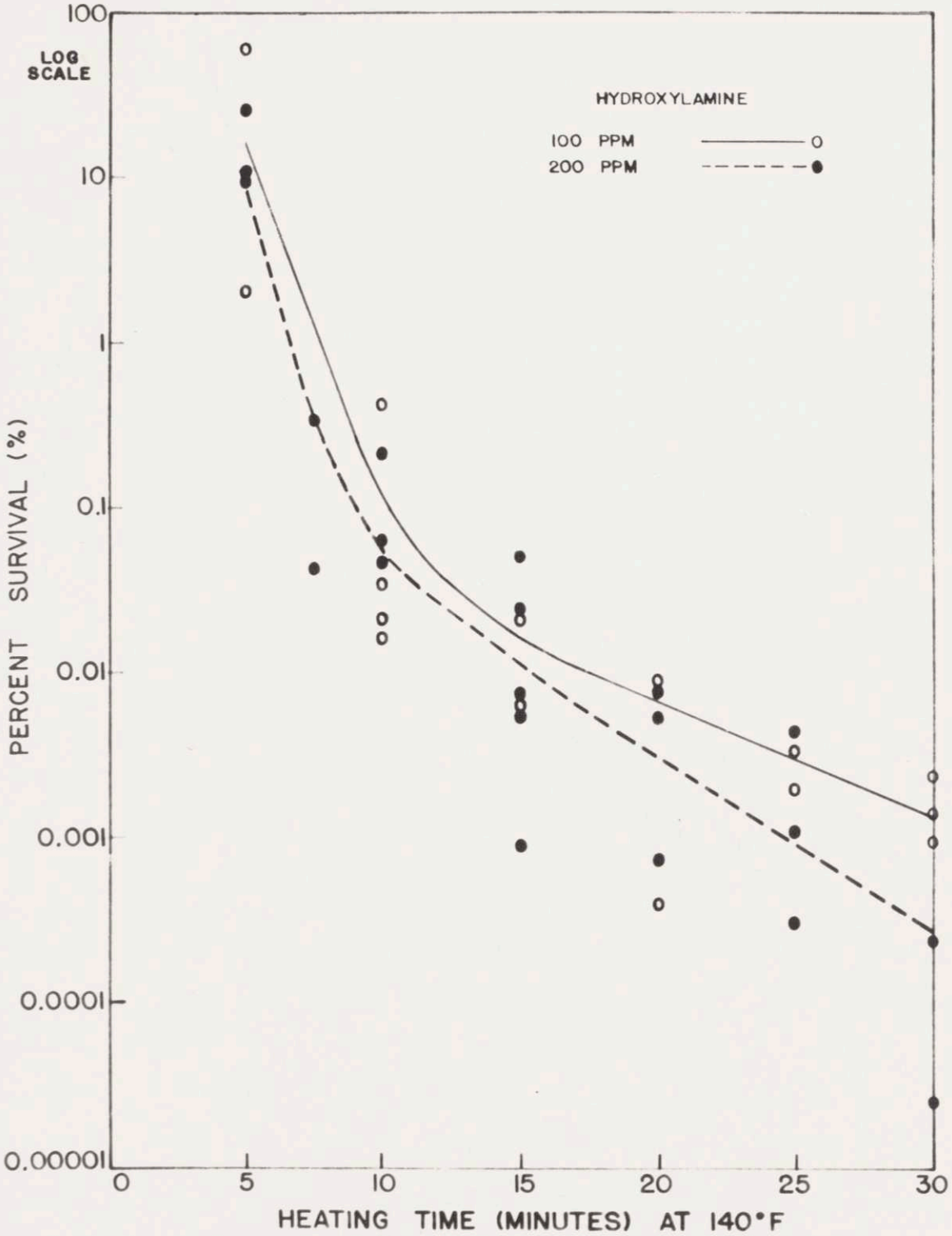

Figure 30. Survival Curves of M. pycenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

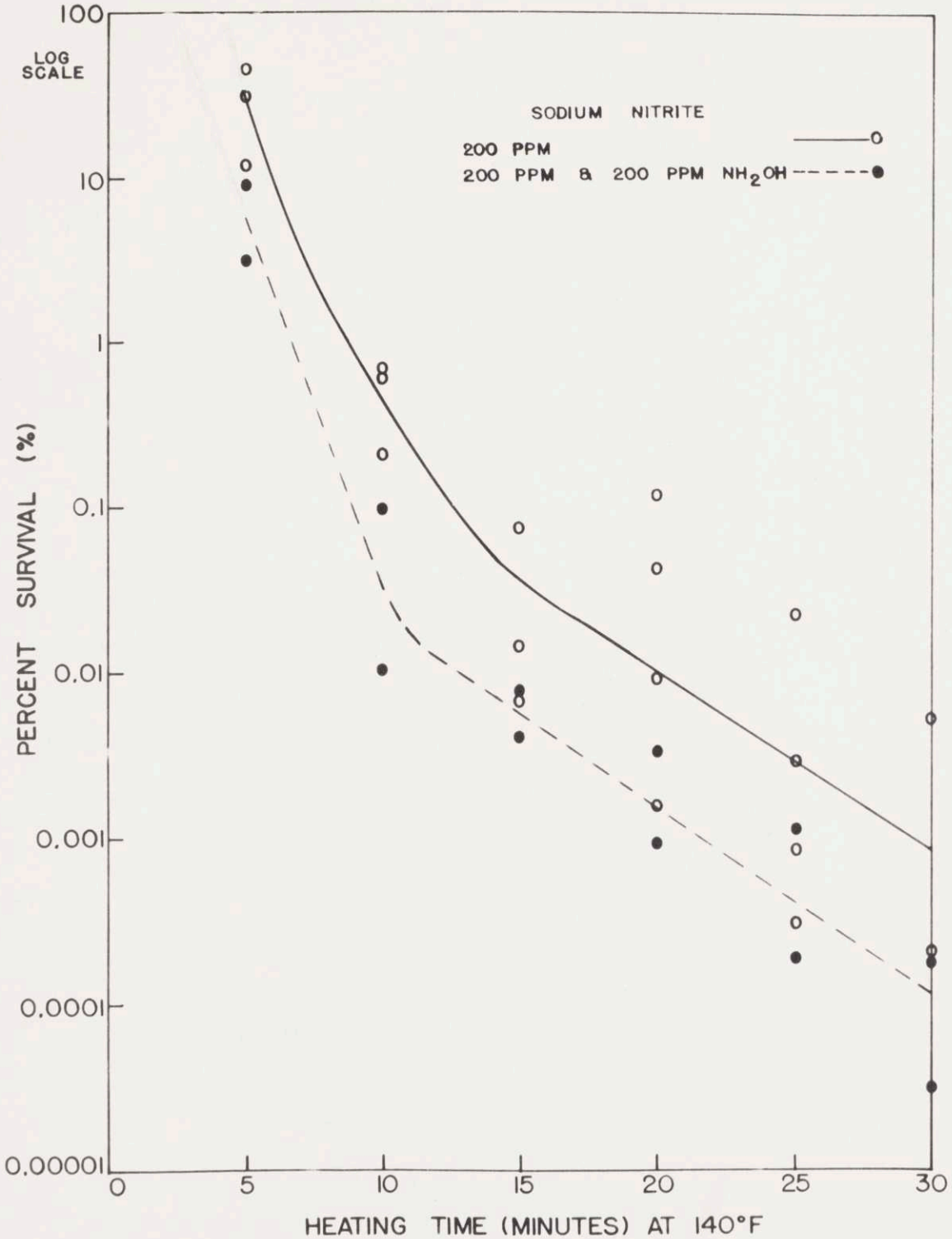

Figure 31. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

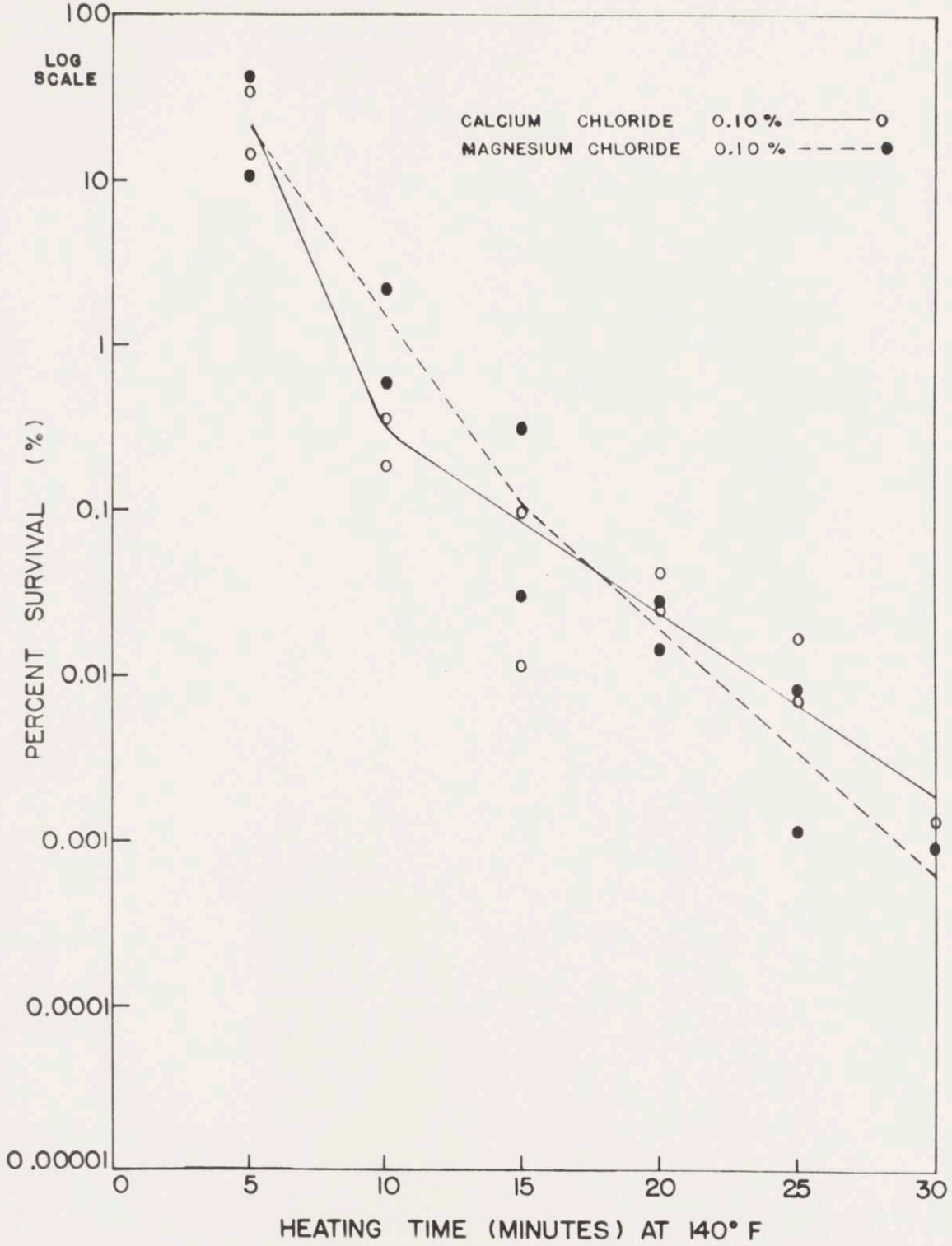

Figure 32. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

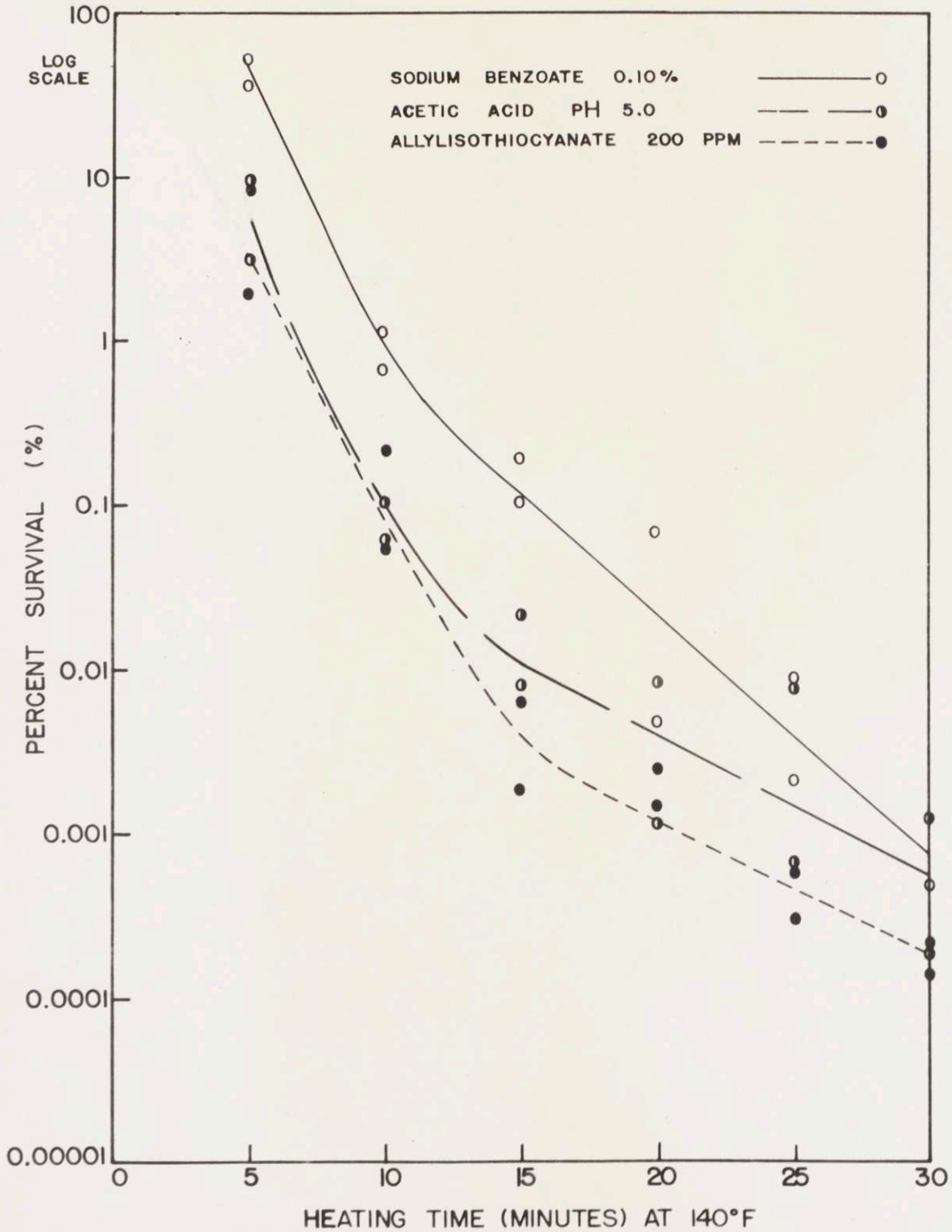

Figure 33. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

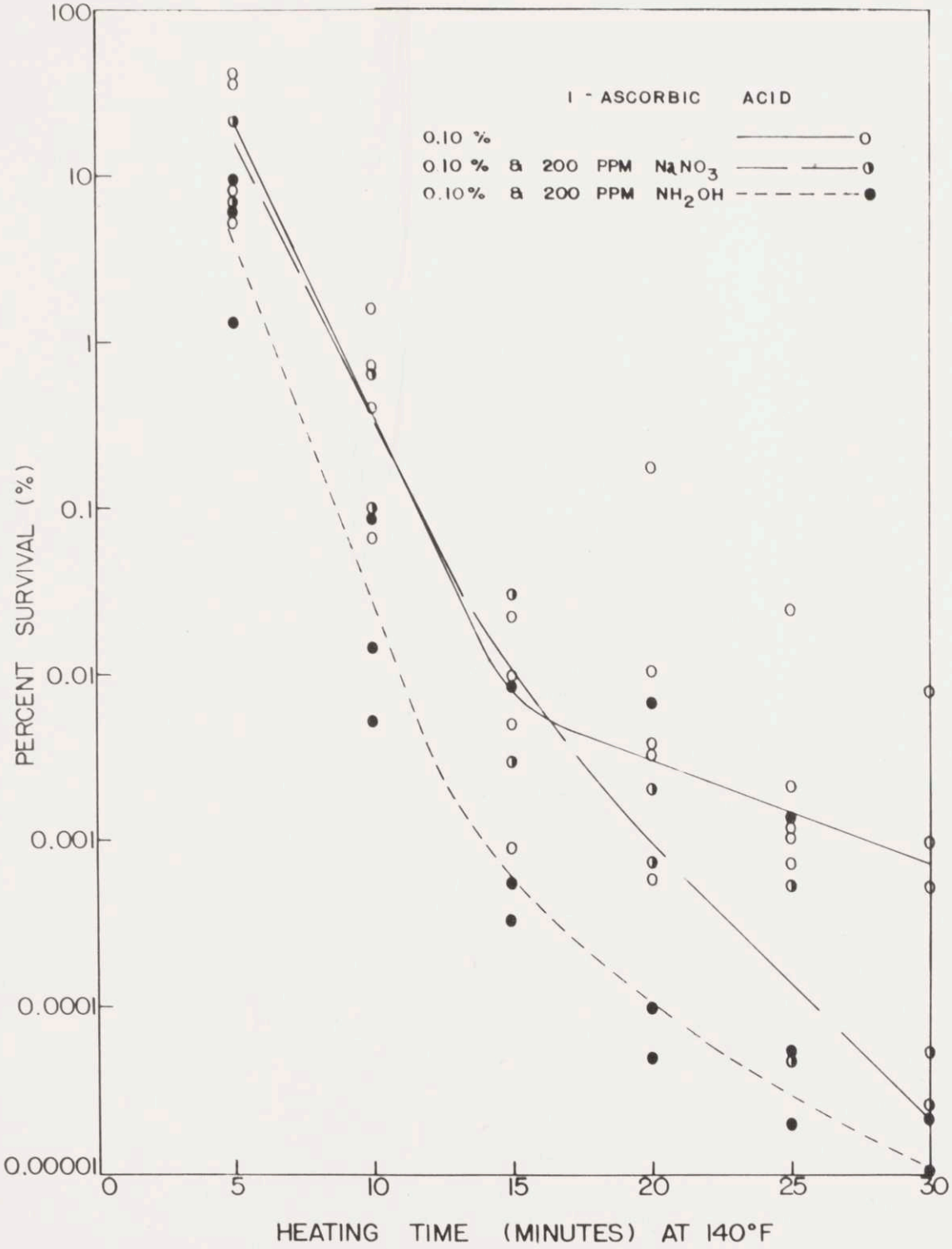

Figure 34. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

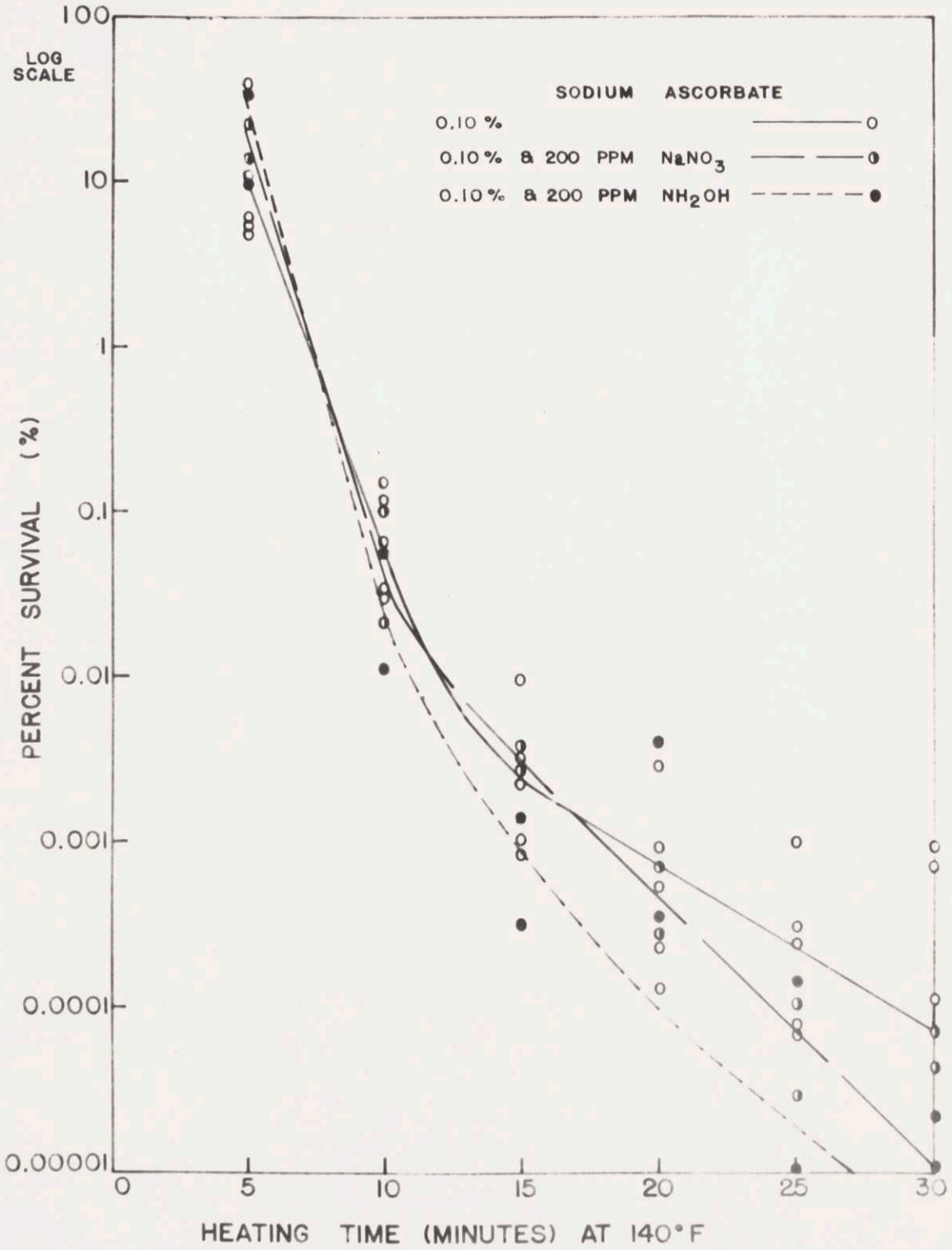

Figure 35. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

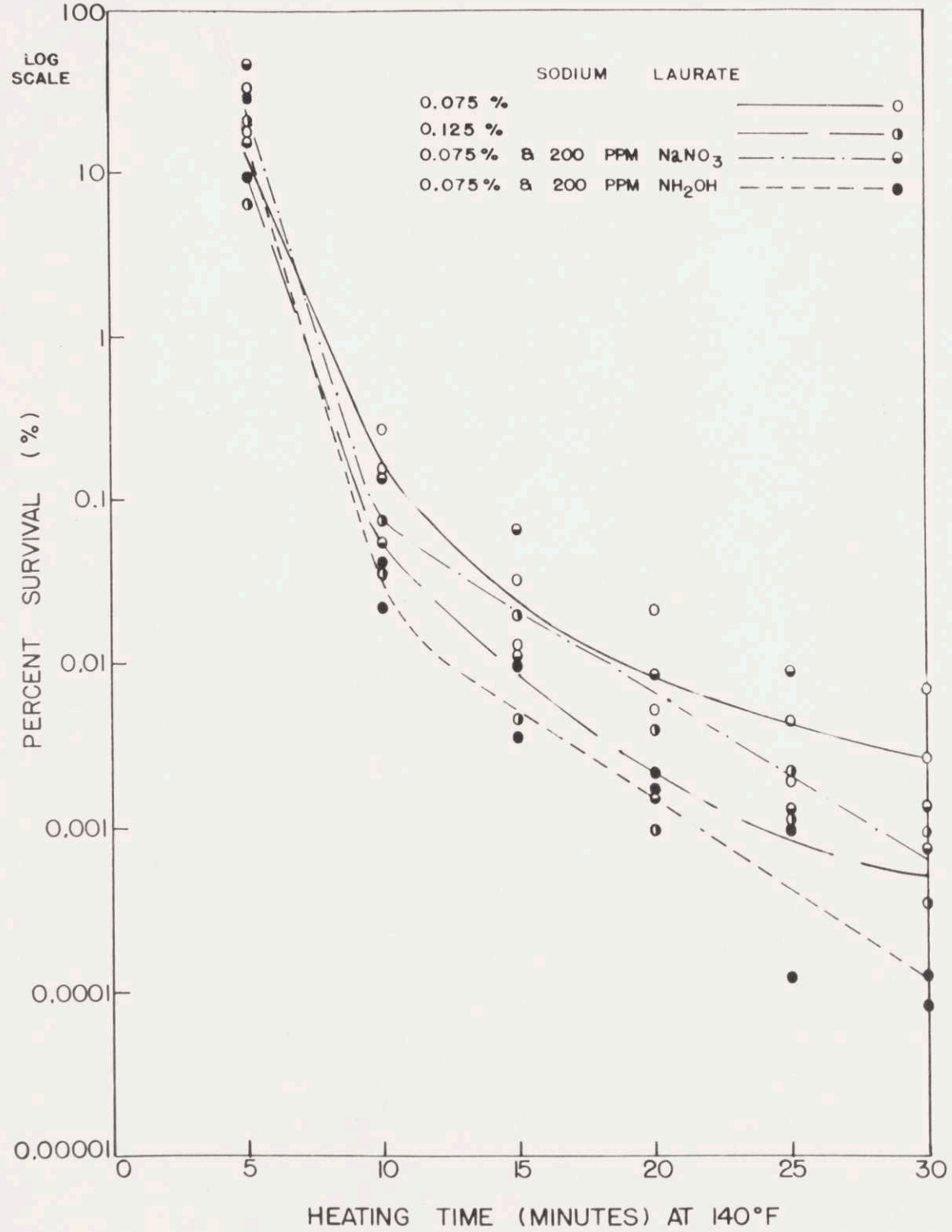

Figure 36. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

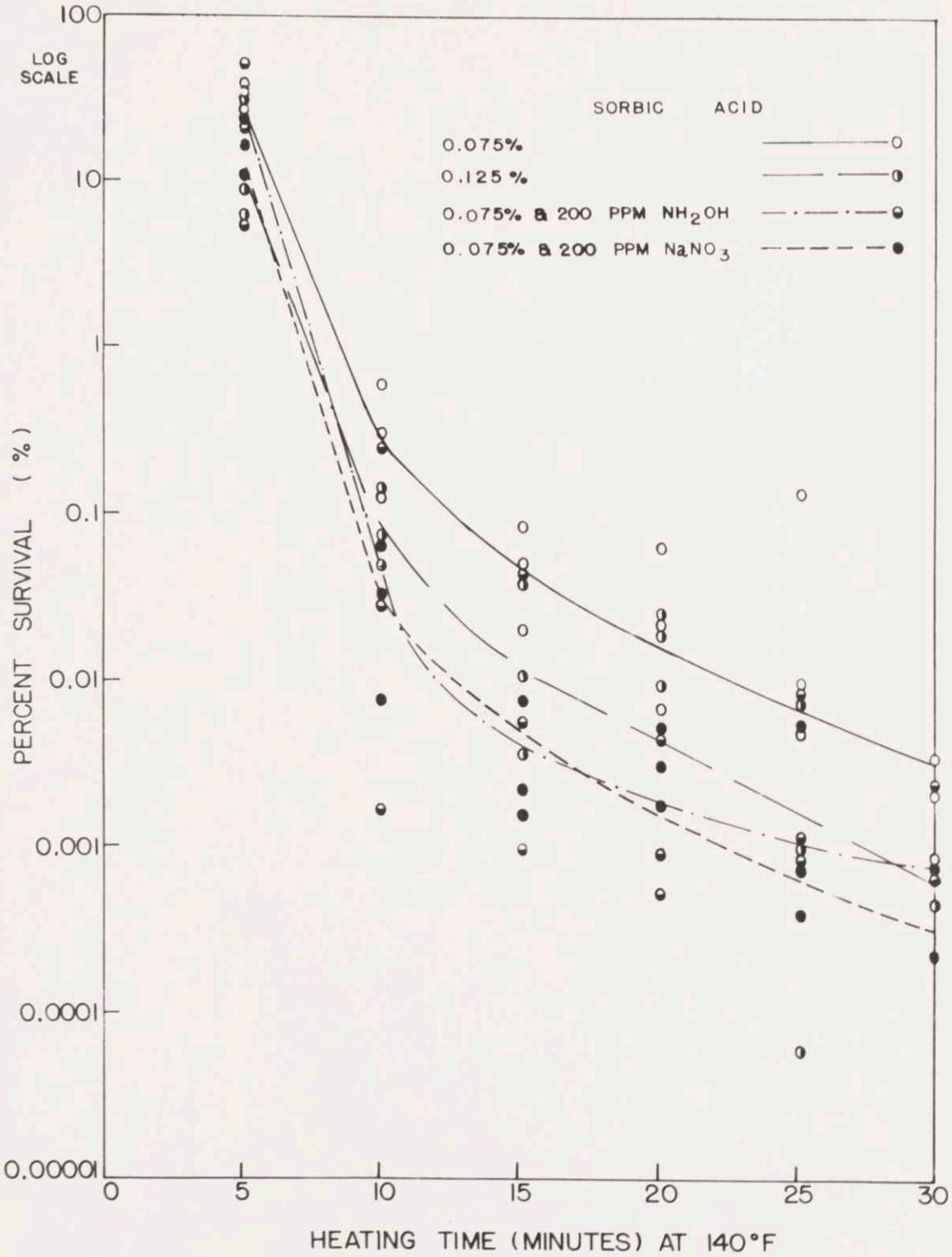

Figure 37. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

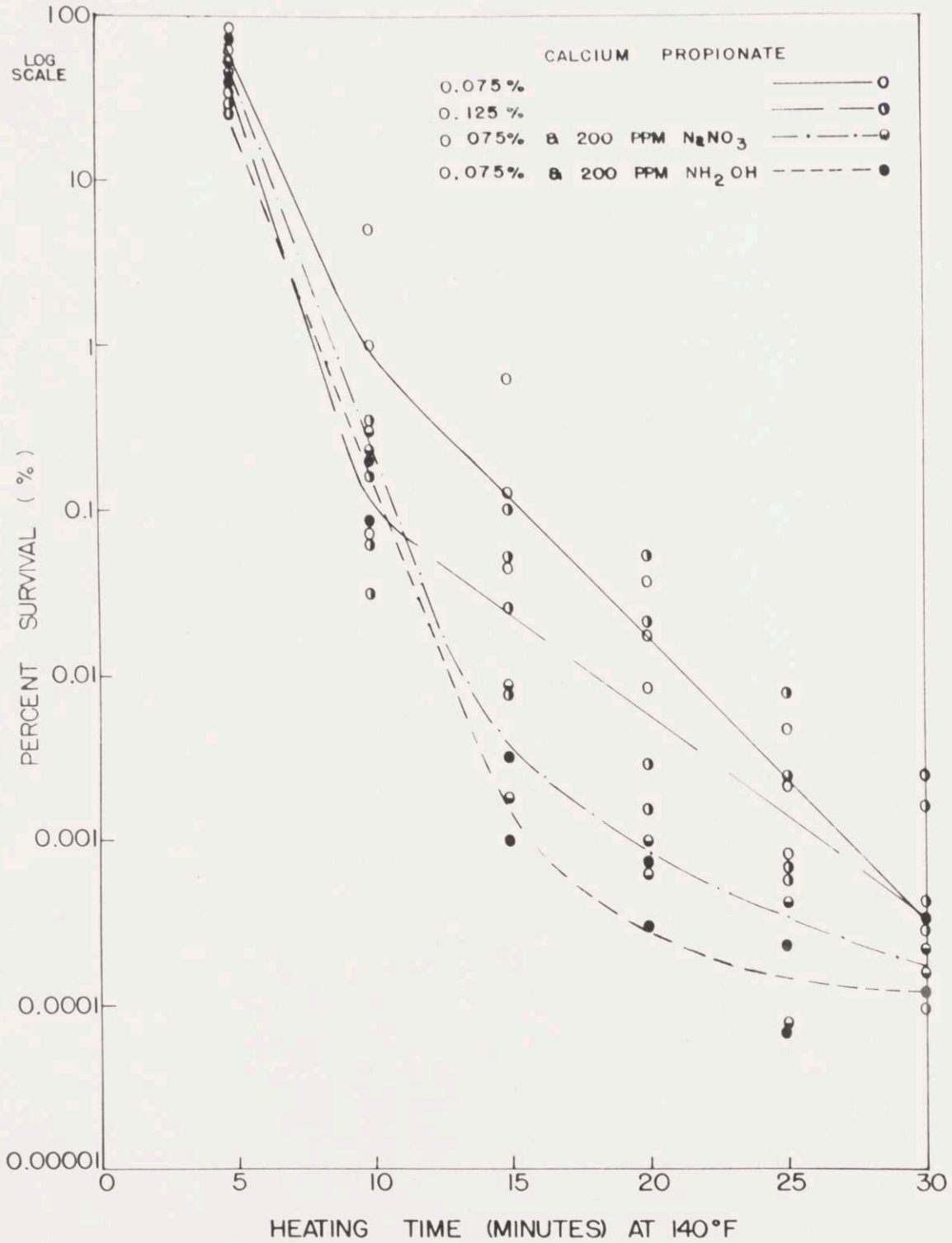

Figure 38. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

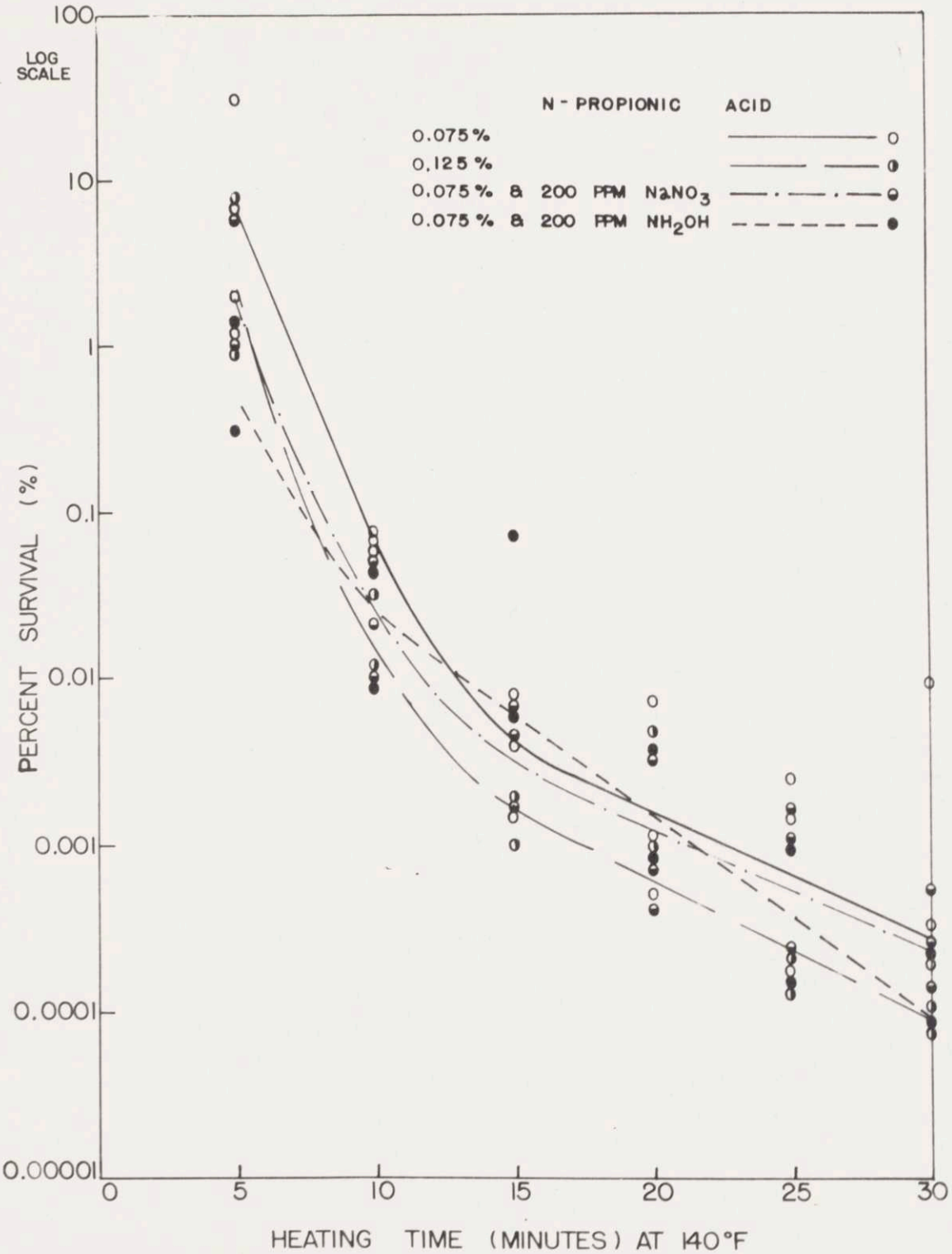

Figure 39. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. Chemical Present in Homogenate.

2. Antibiotics

The survival curves for the four antibiotics are shown for both ham and roast beef homogenates in Figures 40 to 55.

The concentrations used are based on the allowance of the maximum concentration permissible in uncooked poultry, which for Aureomycin and Terramycin is a residue of 7 ppm. The dips used commercially have a concentration of 10 ppm. For this reason the 10 ppm level was selected. One ppm was selected to determine what effect, if any, a minimal level would have on the lethality during heating. One ppm is a concentration of 0.0001%. The selection of 50 ppm was based on the interest in observing the effects of a relatively high concentration, but not a concentration sufficiently great to cause high lethality due to the antibiotic alone. As the curves at 50 ppm show, the selection of this concentration was fortunate. If it had been much higher, it is probable that the lethal effect of heating would have been masked.

The purpose of this work was to show the effects of antibiotics upon the lethal rate at 140°F. Information concerning the effects of antibiotics, themselves, was not the purpose of these experiments. The tubes were heated as quickly as possible after mixing the antibiotic solution and the inoculated homogenate

to minimize the effect due to the antibiotic alone. The elapsed time from preparation of tubes to the start of the heating period was only a few minutes.

The standard curves for both ham and roast beef were those used for chemical effects. These control curves had no antibiotic or chemical added to the homogenate, only 0.5 ml of sterile, distilled water. The curves are shown in Figures 16 and 29.

Although the standard curves are described by straight lines, almost all of the curves obtained with homogenates containing 1 and 10 ppm antibiotic show non-linearity. There was an initially rapid lethality (steep slope), followed by a lessening of the lethal rate during approximately the second half of the 30 minute heating period. This may be the result of inactivation of the antibiotic during the heating period. It is conceivable that the first effect is due to a combination of heating and antibiotic action, and the second is an effect of heating alone. The only exception to the non-linearity of these curves is that of 10 ppm of Aureomycin in roast beef homogenate. In this case, the survival curve appeared to follow a straight line.

At 50 ppm, all curves, with the exception of that of Terramycin in roast beef, are best described as straight lines. The exception showed a curved effect, but the change of shape was not as severe as those seen at lower

concentrations. The absence of any demonstrated antibiotic destruction effect during heating at 140°F. is probably best explained by the high initial concentration of antibiotic. If there was any destruction, and it is logical to assume that there was some, there still remained a sufficient amount to have a noticeable lethal effect. If, for example, 50 per cent were destroyed between 10 and 15 minutes of heating, an initial concentration of 50 ppm would then be reduced to 25 ppm. This would be 2.5 times as much as 10 ppm, at which concentration, a very significant lethal effect occurs. Not only was there sufficient activity to continue influencing the lethal rate, but the end point of 99.99999% destruction was reached well within the 30 minute heating period. As a result, should the hypothetical 50 per cent be destroyed within 10 to 15 minutes, it would only matter slightly because the end point was reached in this time.

On the other hand, referring to the lower concentrations, 50 per cent of 1 ppm is only 0.00005 per cent. Thus if 1 ppm slightly accelerated the lethal rate of heating at 140°F, 0.5 ppm might not have any observable influence, and the last few points on the curve at 20 to 30 minutes might be of the same order as the lethal effect of heating alone. There might be a slight dif-

ference, however, even should a given antibiotic have no effect toward the end of the heating. This might be based on a lethality associated only with the antibiotic, destroying cells,which with heating alone, would not be destroyed.

One effect of 50 ppm of added antibiotic is the reduced lag time after heating has begun, before lethality occurs. The curves at this concentration show that in some cases, the destruction of Micrococcus pyogenes, var. aureus occurred without any heating. This varied with the antibiotic and substrate used. By extrapolation of the curves to 0 minutes of heating, it is possible to estimate the amount of lethality occurring in the non-heated sample. The sample count at zero time is assumed for calculation purposes to represent 100 per cent survival.

The extrapolated values, indicating an estimated bacterial destruction in the unheated samples at a level of 50 ppm of antibiotic were as follows:

Antibiotic	Ham	Roast Beef
Aureomycin	0% *	67%
Terramycin	20	0 **
Nisin	82	99.15
Subtilin	85	99.20

* The extrapolated values showed no lethality in the unheated samples. Lethality began at 1.5 minutes.

** Similar to above. Lethality began at 1.6 minutes.

With the exception of Terramycin, the reduction in the unheated samples would tend to show a difference in response between ham and roast beef substrates. Greater lethality seemed to occur with roast beef.

This difference in response held true through the entire range of antibiotics at the three concentrations, and followed the same pattern observed with chemical additives. The statement of greater lethality is based on the respective lethal lag times, the relative slope and position of the curves, and the extent of survival at the end of the 30 minute heating period. The greater lethal effect in roast beef varied in magnitude but was consistent.

It is difficult to quantitate the altered lethal rates due to the addition of antibiotics. The method was designed for screening antibiotic effects. It may be said that during heating, nisin and subtilin had similar effects on the destruction of Micrococcus pyogenes, var. aureus in ham, which were greater than that of either Terramycin or Aureomycin. In roast beef, however, 1 and 10 ppm of Aureomycin had a distinctly greater effect on the lethal rate than any of the other three antibiotics at these concentrations. Nisin and subtilin had substantially greater effects at levels of 50 ppm.

The effects of antibiotics upon the rate of destruction of Micrococcus pyogenes, var. aureus at 140°F are noticeable. Low concentrations were shown to be effective. The immediate use of either Aureomycin or Terrramycin would depend upon proof that the conditions of processing would destroy all detectable quantities of these materials. Use of the non-approved antibiotics, subtilin and nisin,would require much more toxicological and chemical data, as well as the development of conclusive assay methods for the detection of small quantities in foods.

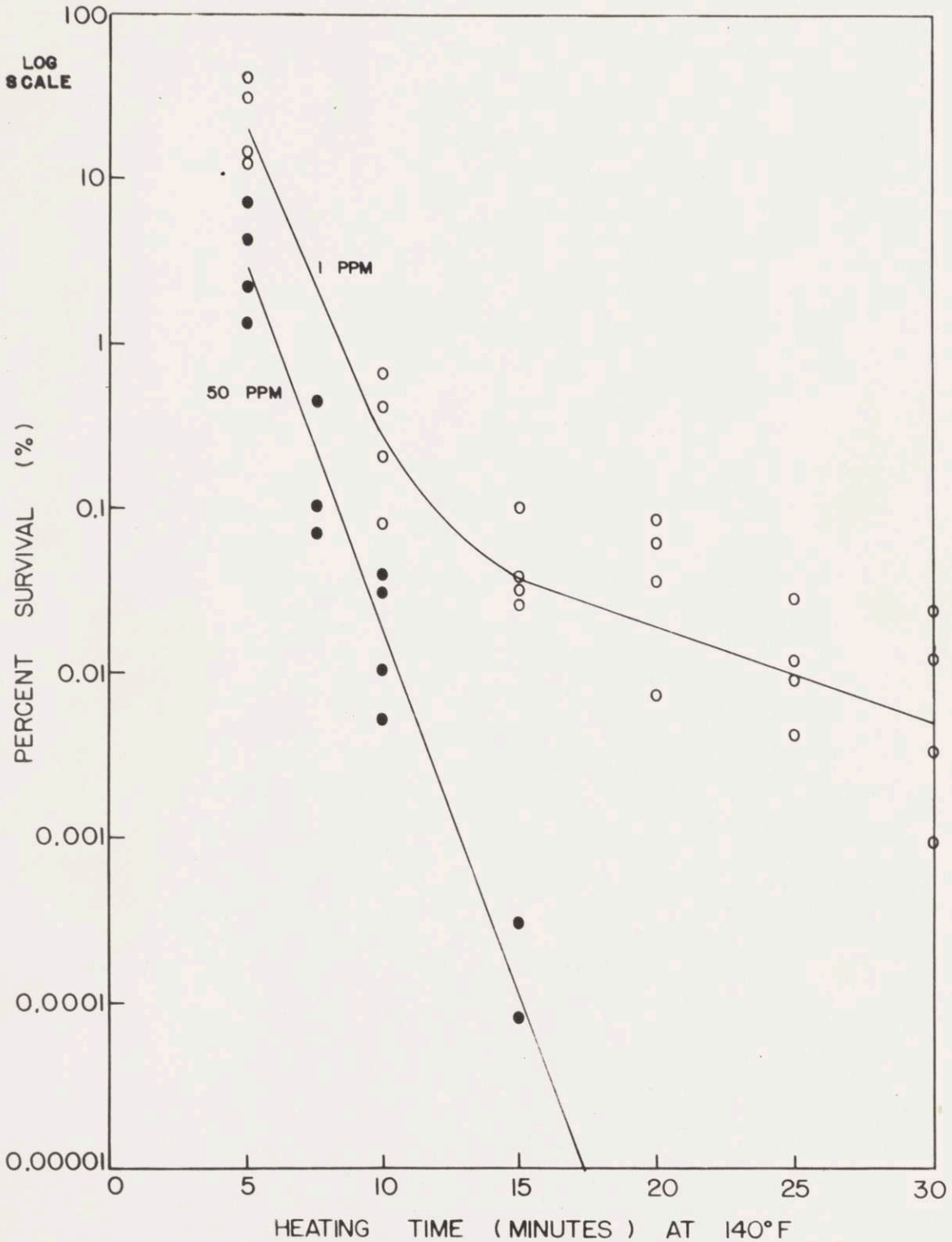

Figure 40. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 1 and 50 ppm Aureomycin present.

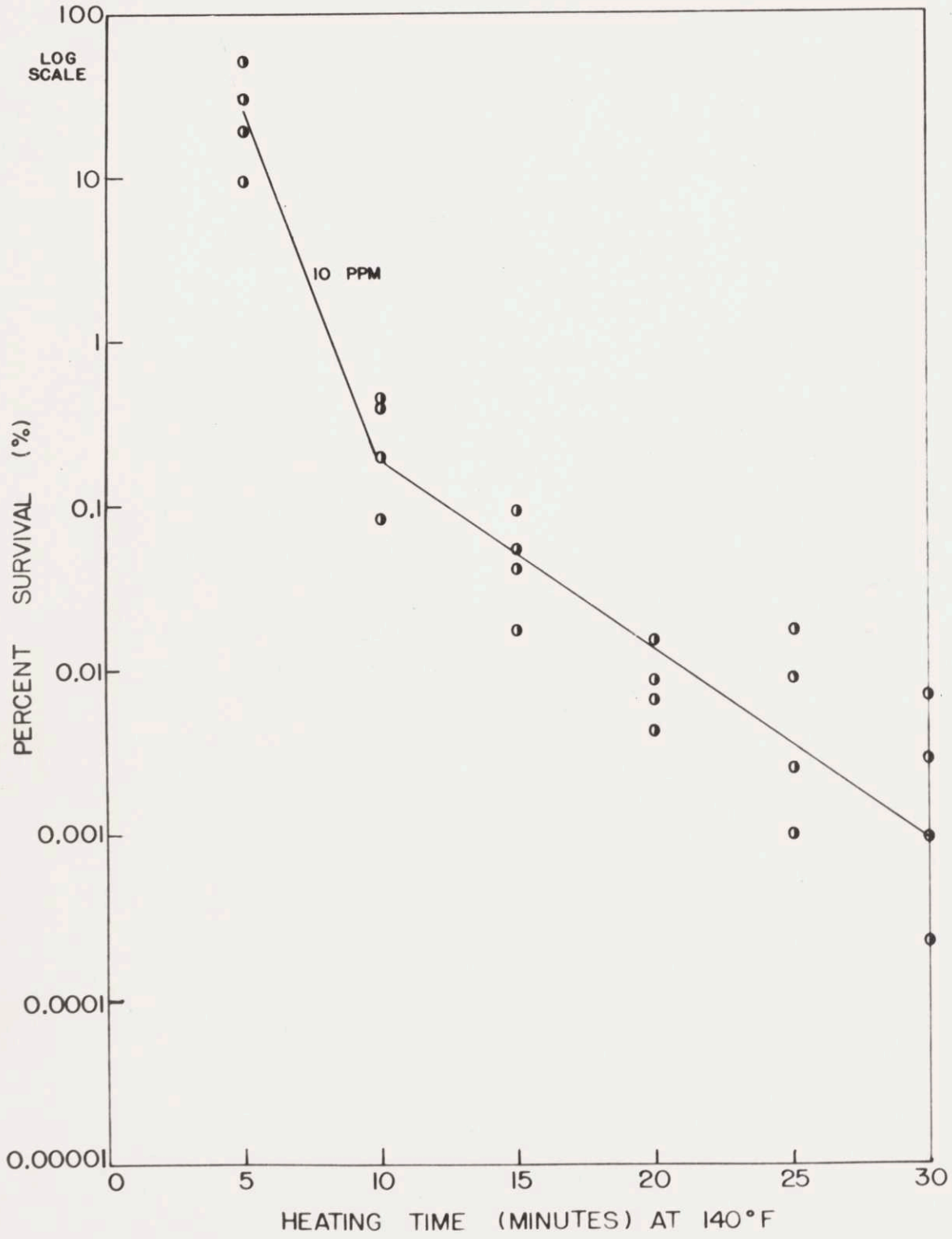

Figure 41. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 10 ppm Aureomycin present.

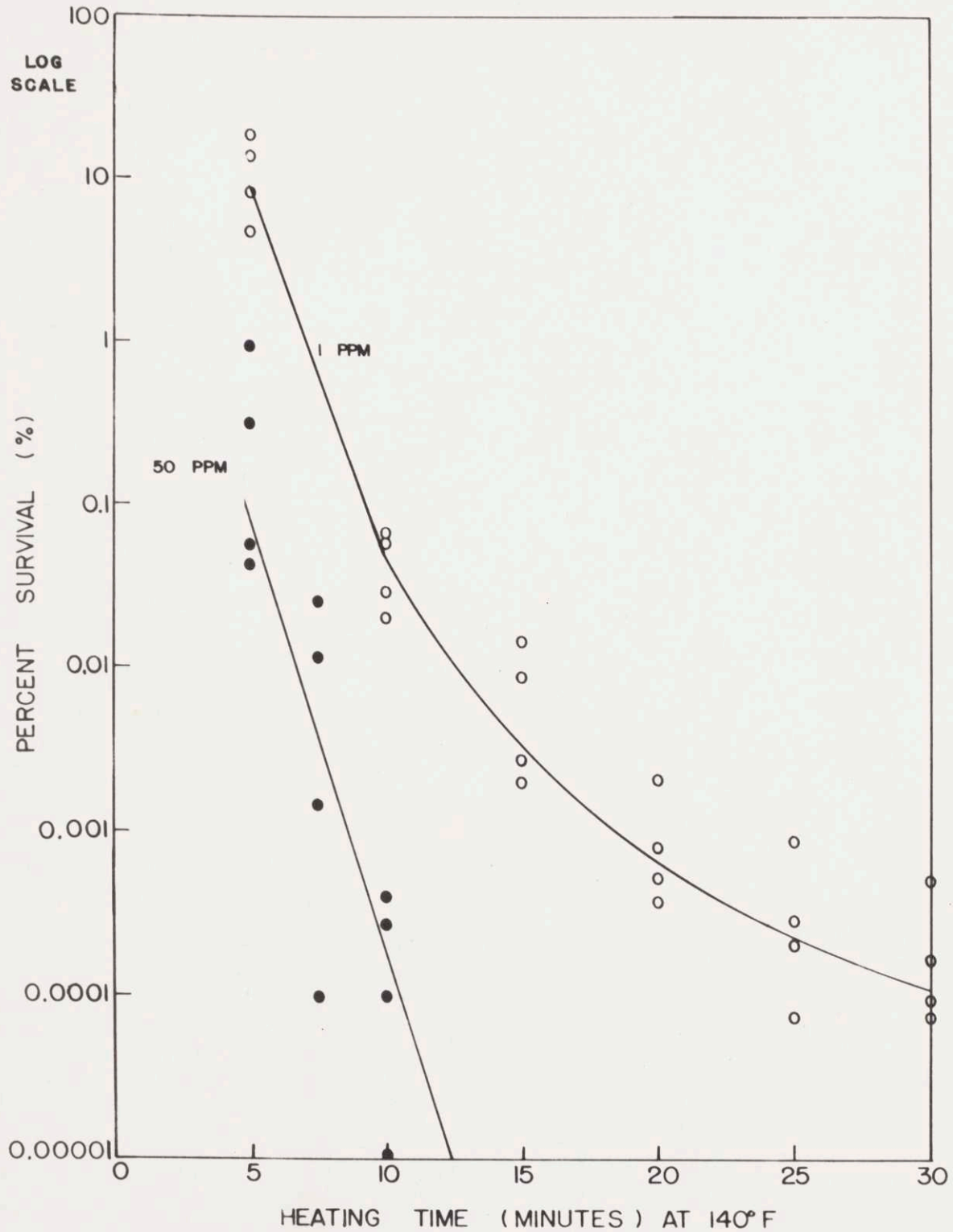

Figure 42. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 1 and 50 ppm Aureomycin present.

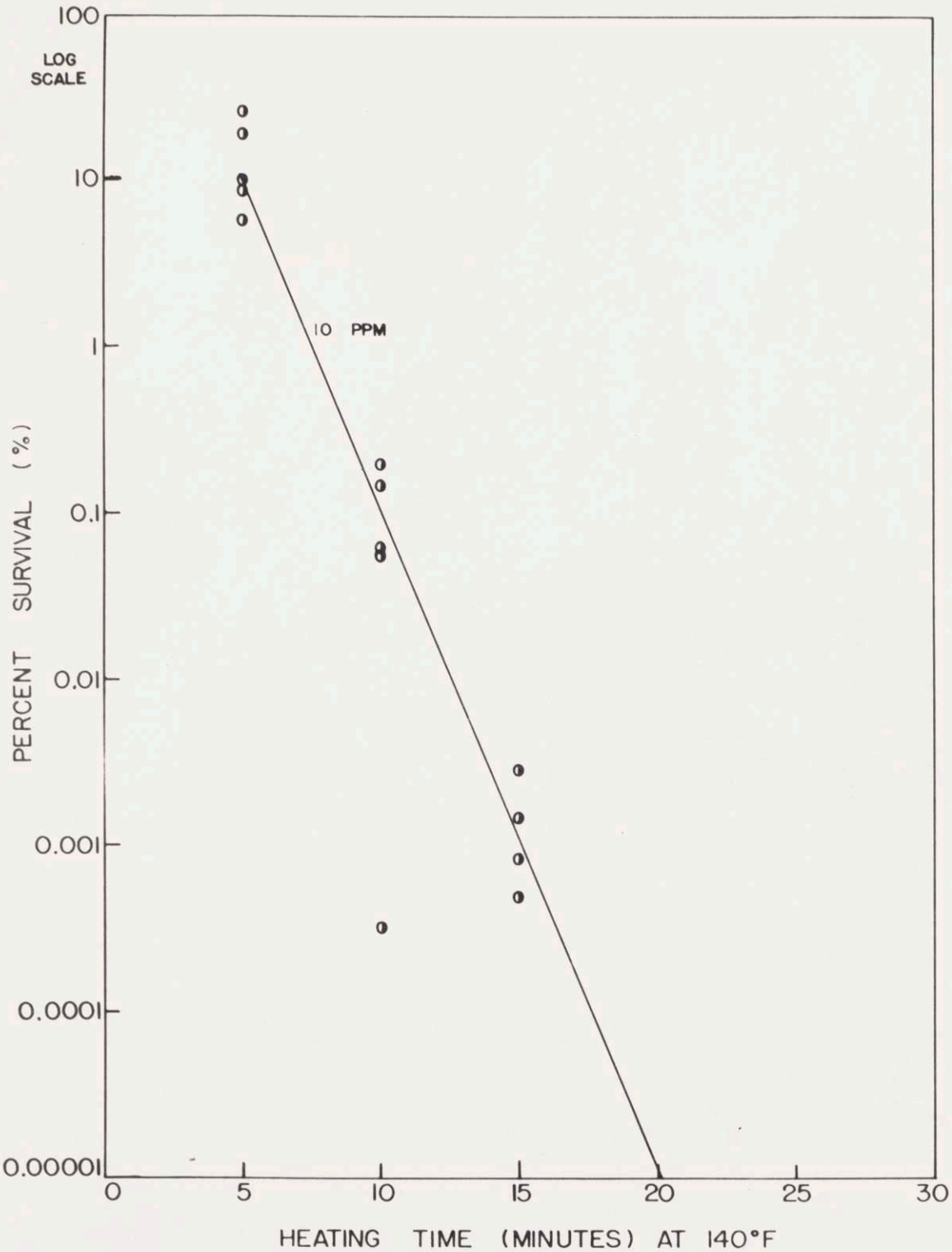

Figure 43. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 10 ppm Aureomycin present.

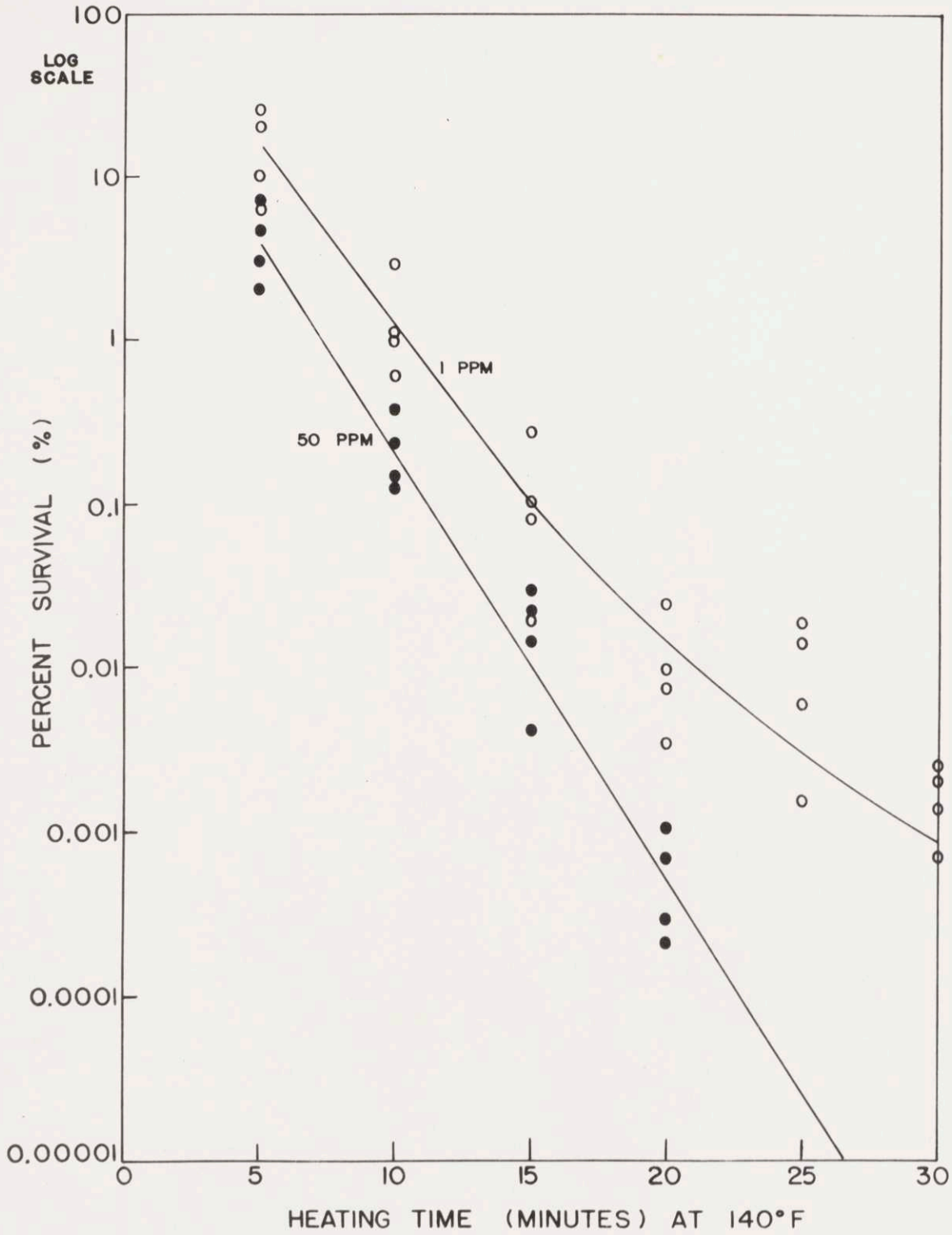

Figure 44. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 1 and 50 ppm Terramycin present.

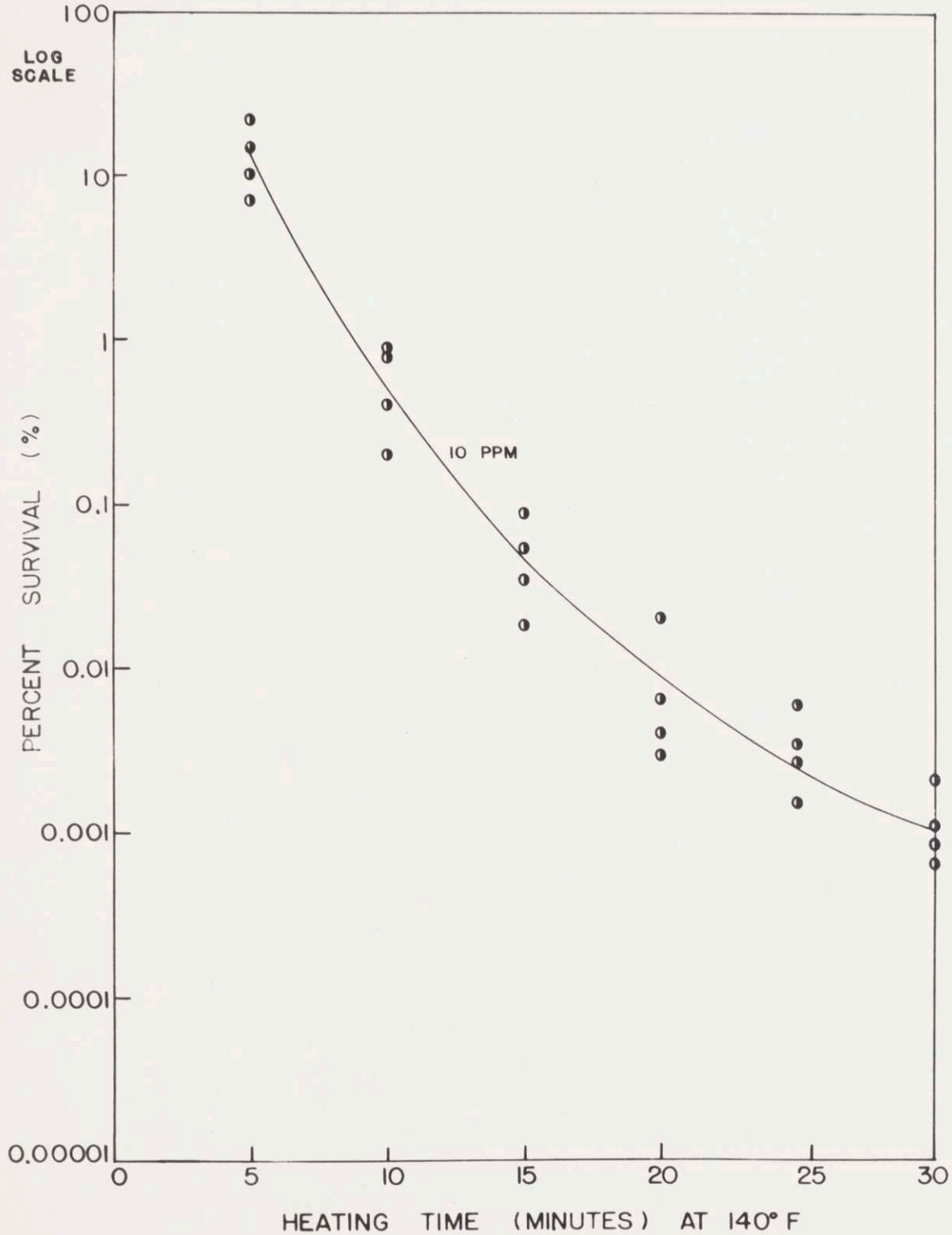

Figure 45. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 10 ppm Terramycin present.

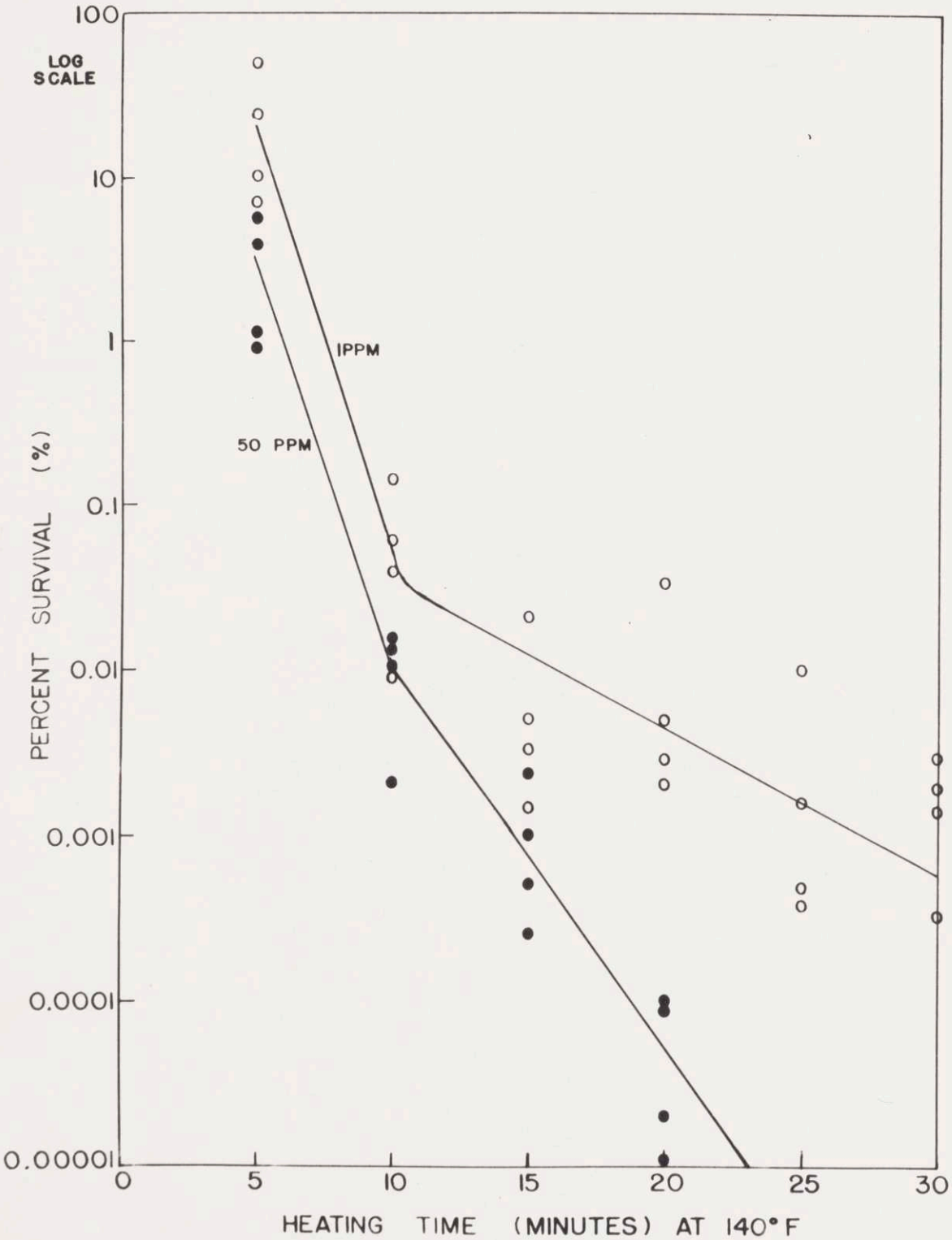

Figure 46. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 1 and 50 ppm Terramycin present.

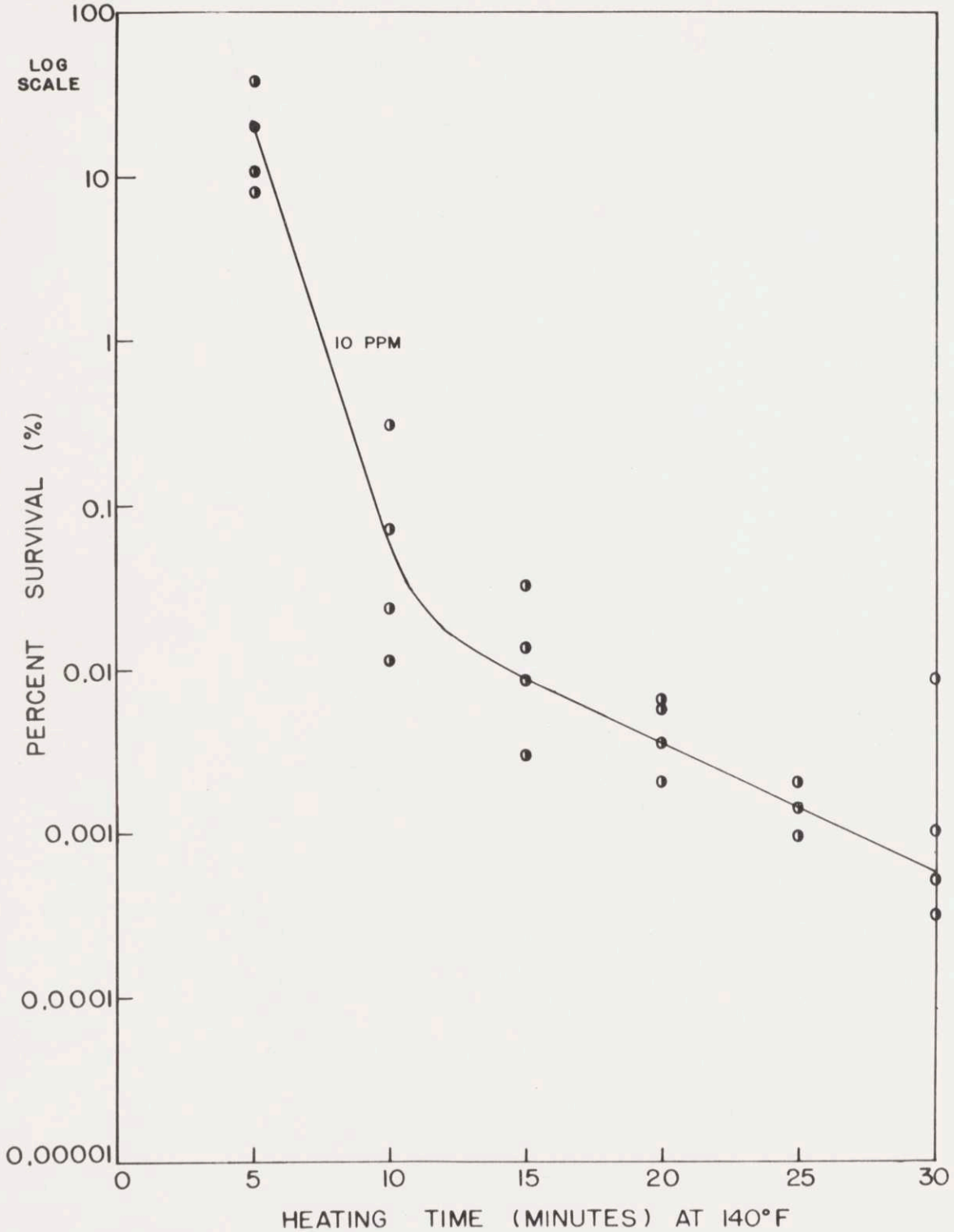

Figure 47. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 10 ppm Terramycin present.

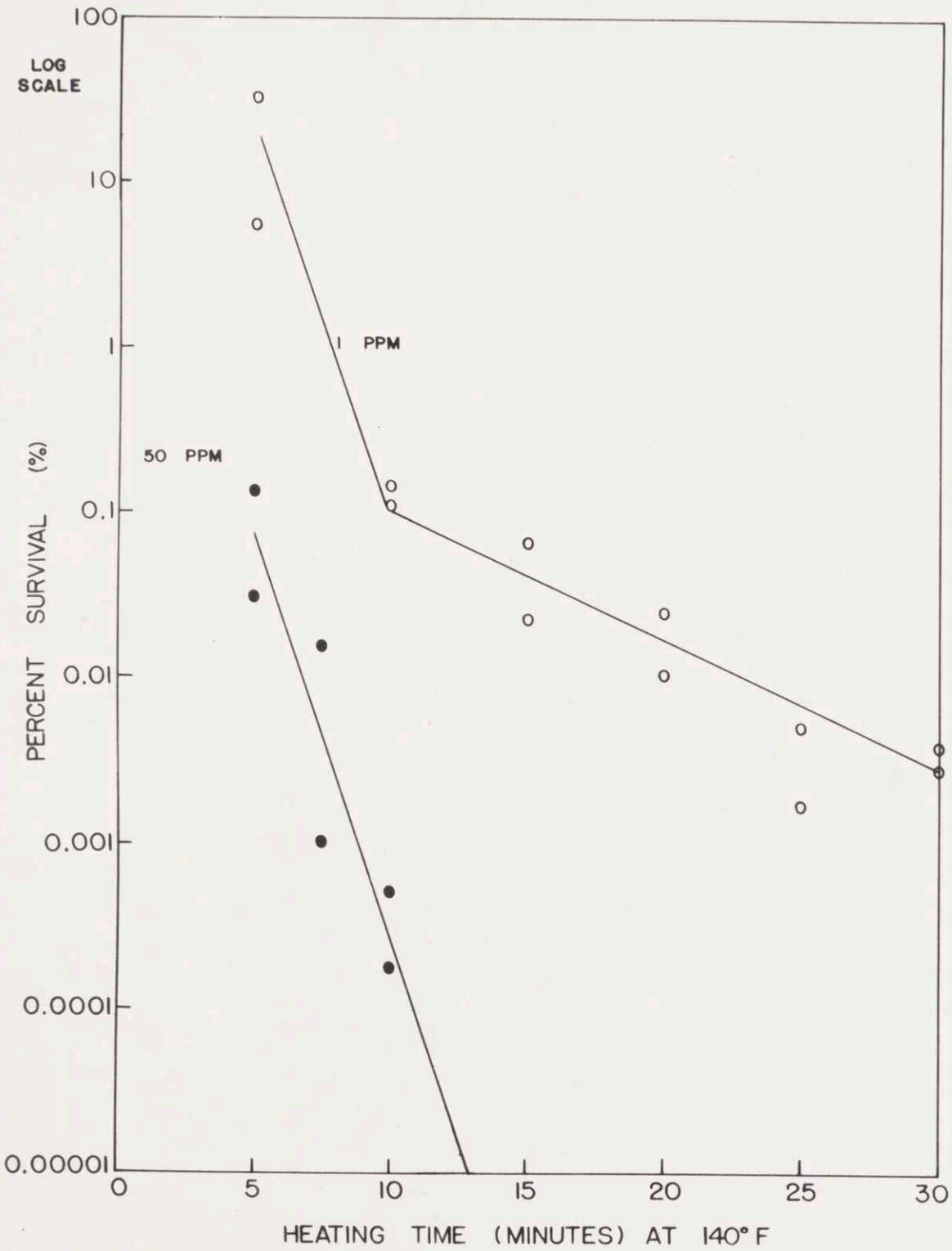

Figure 48. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 1 and 50 ppm Nisin present.

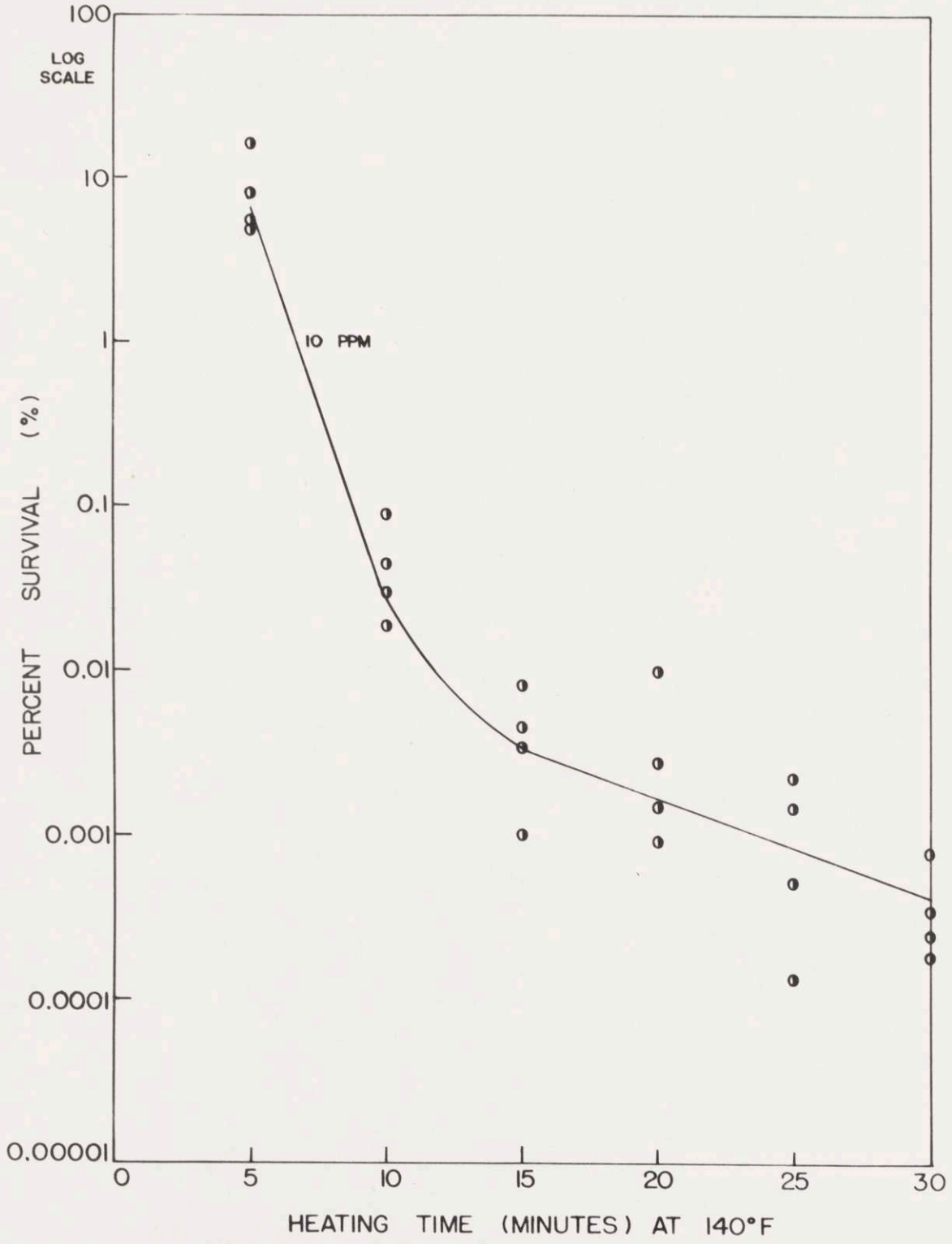

Figure 49. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 10 ppm Nisin present.

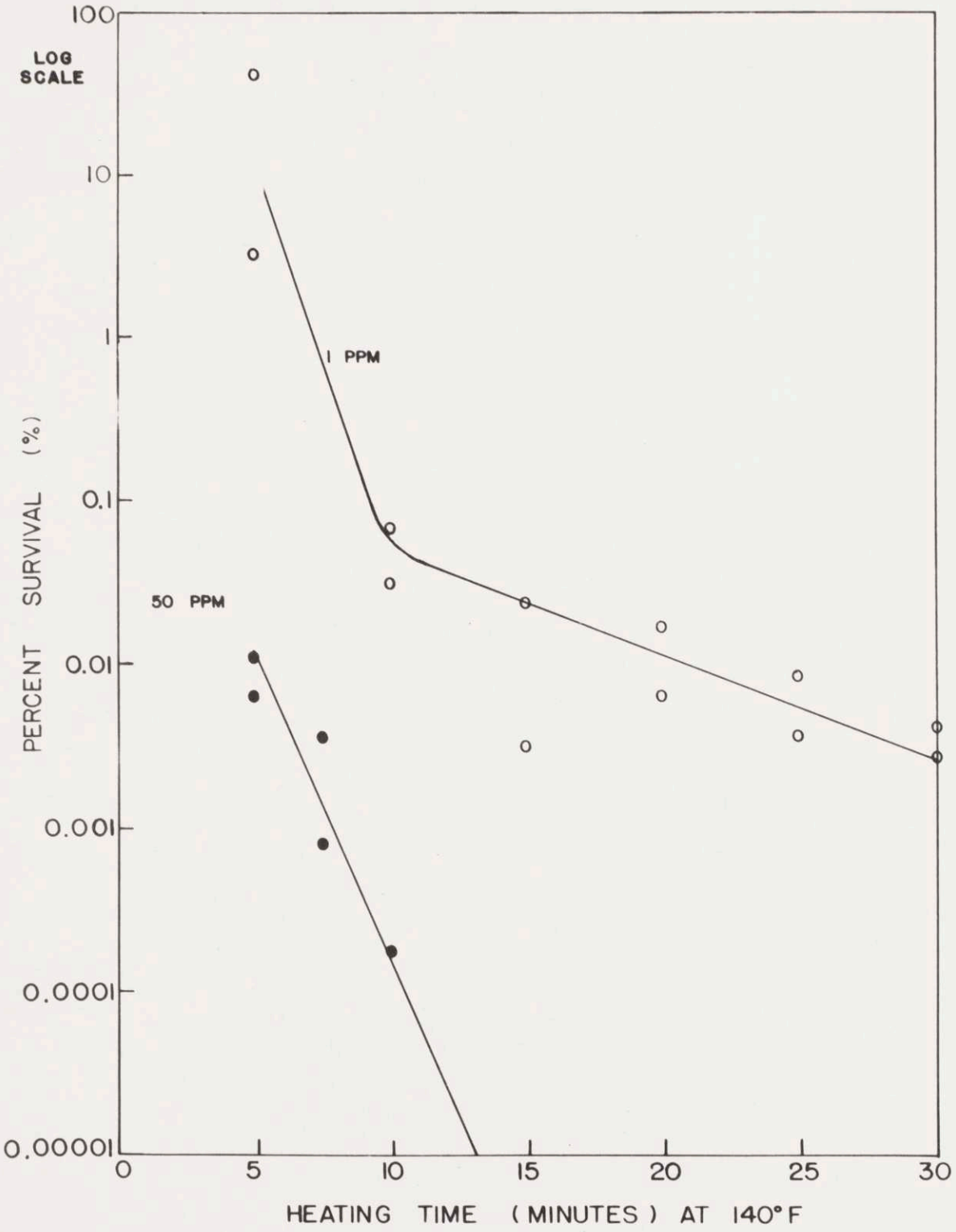

Figure 50. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 1 and 50 ppm Nisin present.

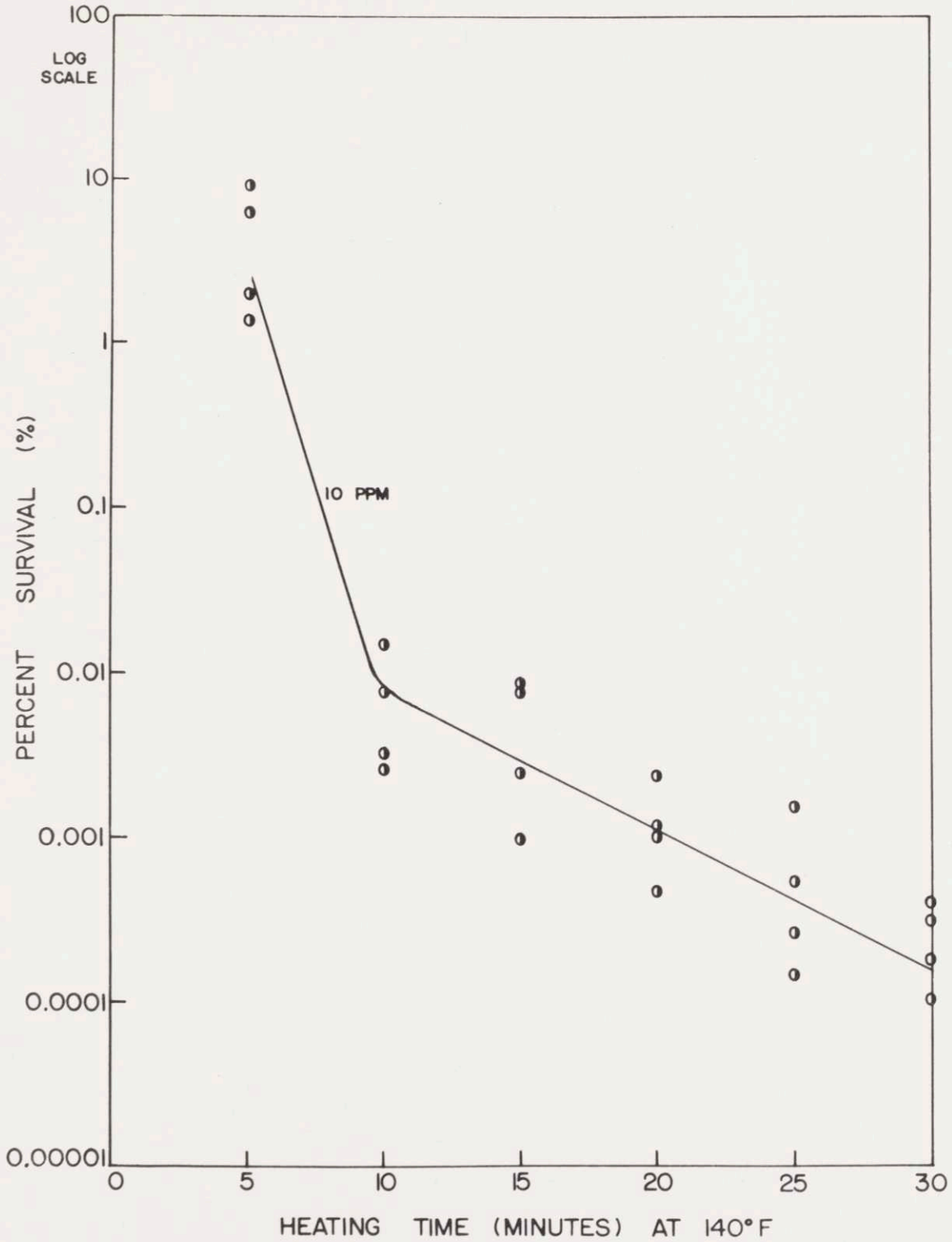

Figure 51. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 10 ppm Nisin present.

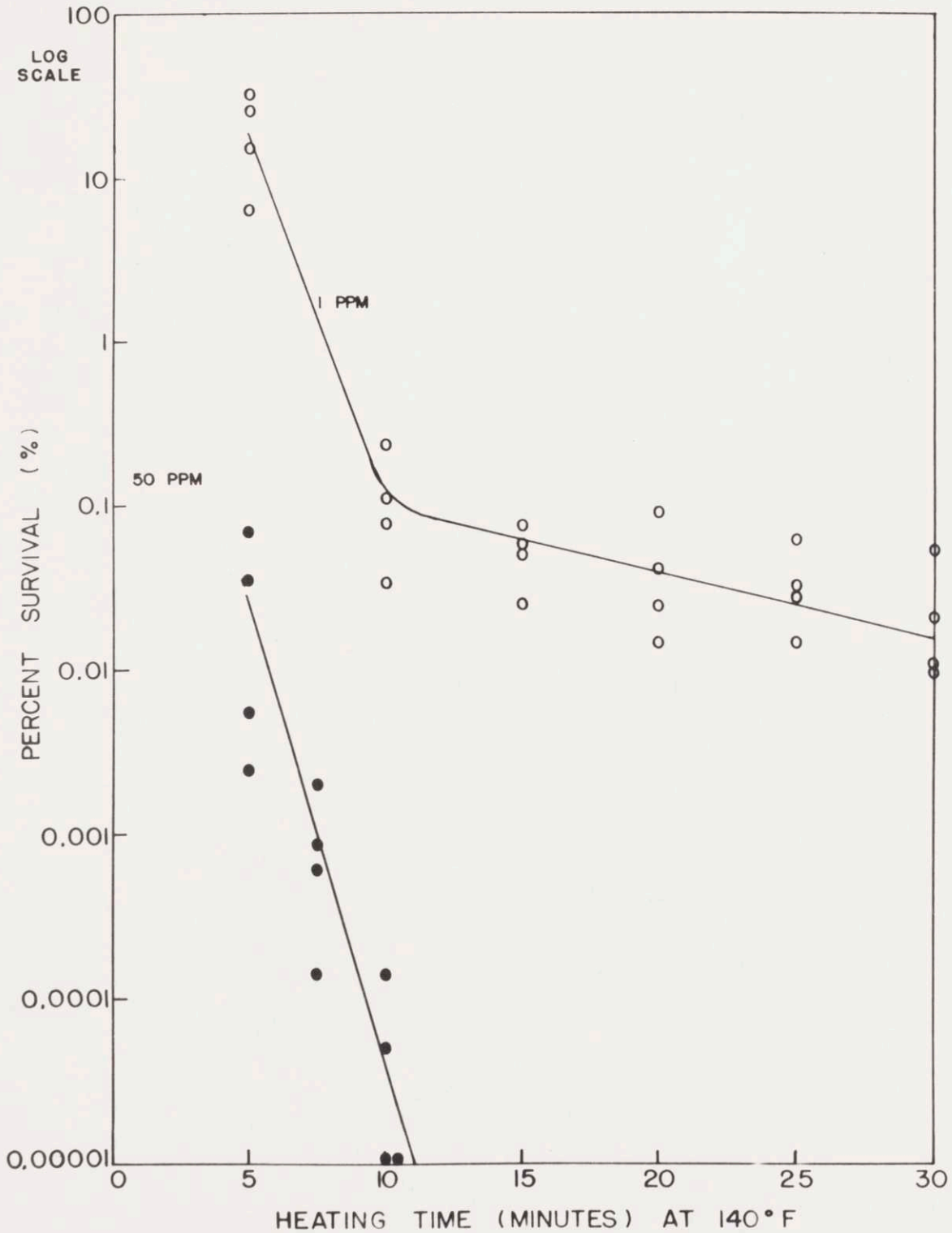

Figure 52. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 1 and 50 ppm Subtilin present.

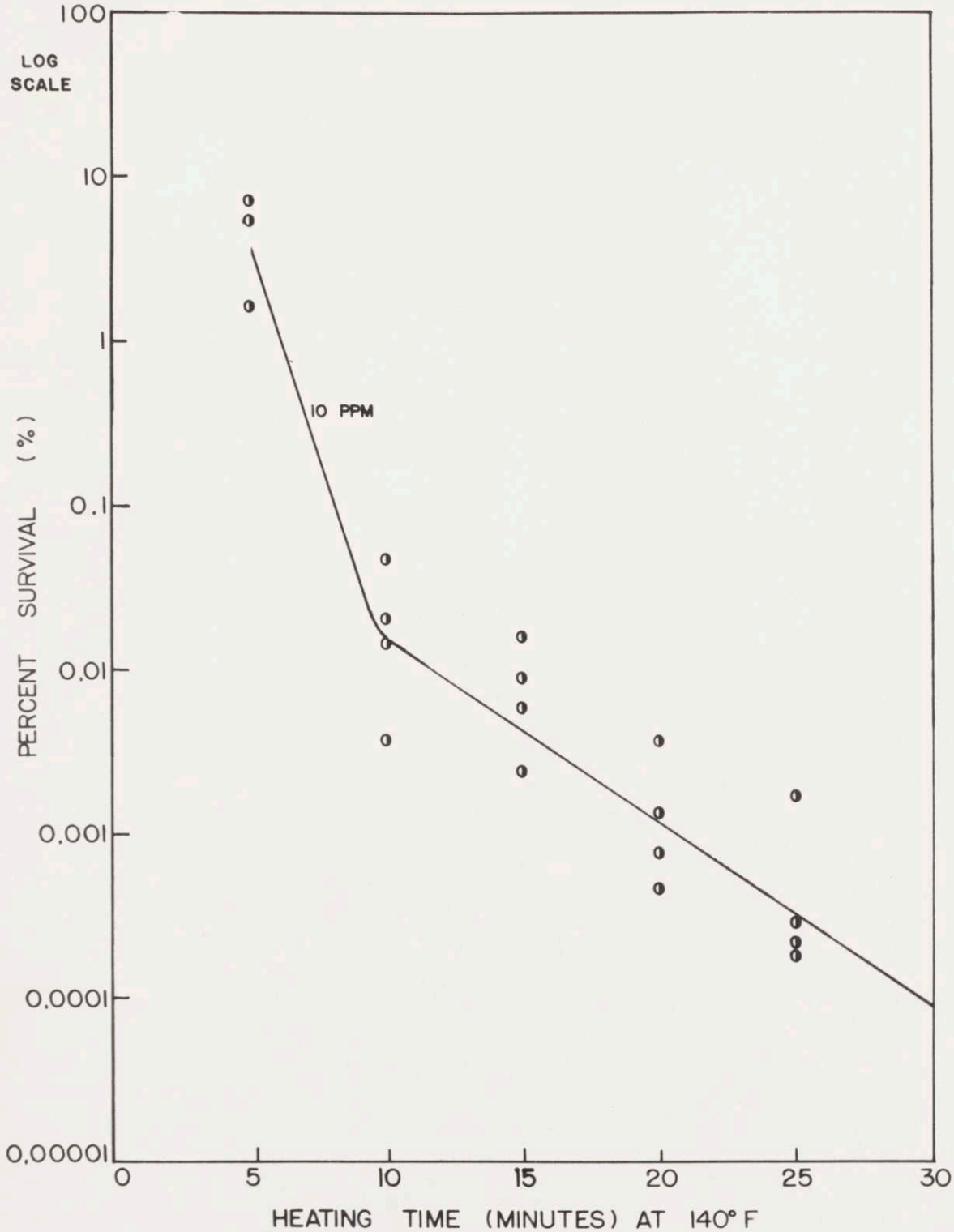

Figure 53. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Ham Homogenate Heated at 140° F. 10 ppm Subtilin present.

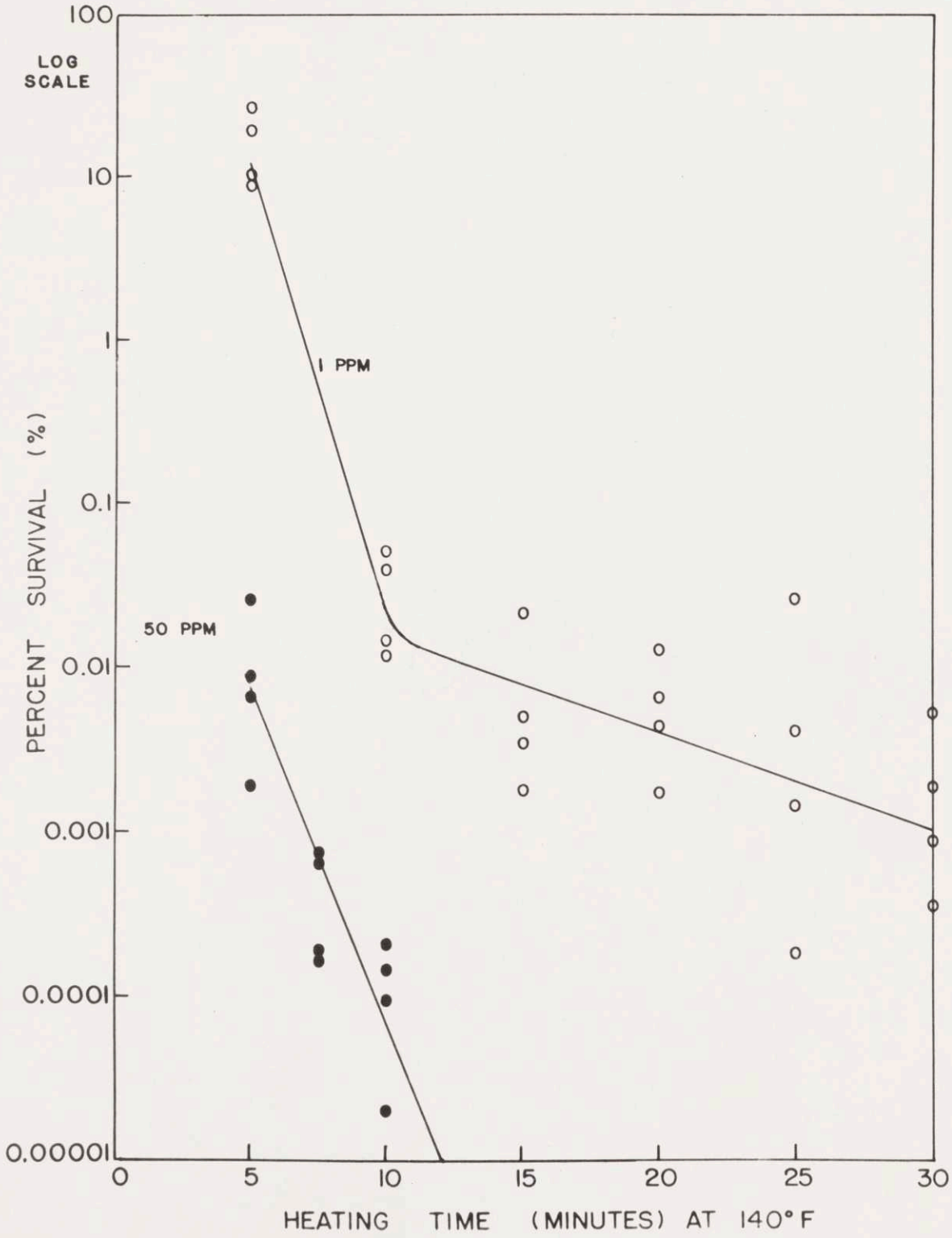

Figure 54. Survival Curves of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 1 and 50 ppm Subtilin present.

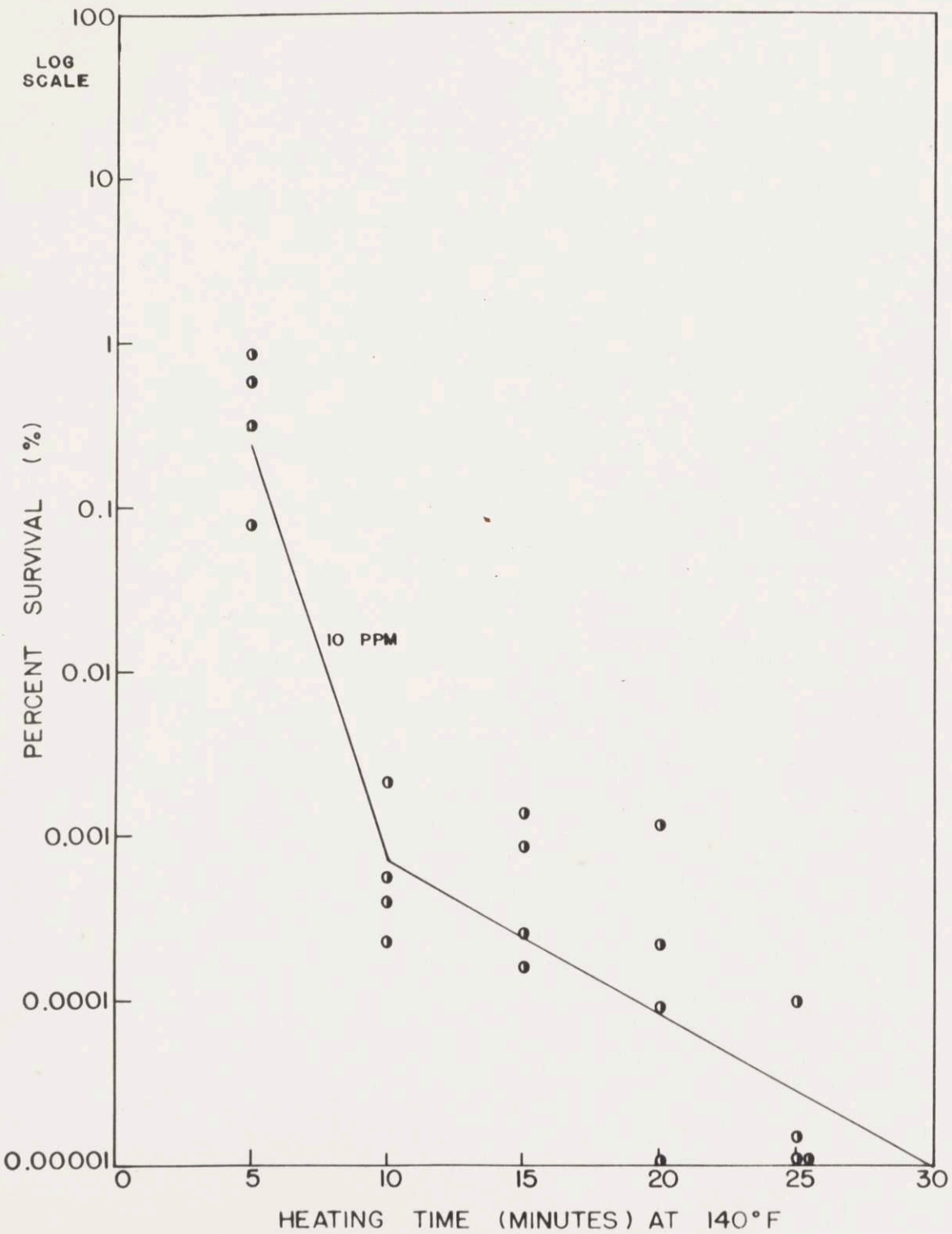

Figure 55. Survival Curve of M. pyogenes, var. aureus, F.D.A. 209 Suspended in 2.5 ml of Roast Beef Homogenate Heated at 140° F. 10 ppm Subtilin present.

VI.

CATHODE RAY IRRADIATION OF ROAST BEEF AND HAM SLICES INOCULATED WITH MICROCOCCUS PYOGENES, VAR. AUREUS

VI. CATHODE RAY IRRADIATION OF ROAST BEEF AND HAM SLICES INOCULATED WITH MICROCOCCUS PYOGENES VAR. AUREUS

A. EXPERIMENTAL PROCEDURE

1. Equipment

Radiation was carried out with electrons from a General Electric Resonant Transformer at an electron (Beta-ray) energy level of 1 M.e.v. This apparatus is located in the Department of Food Technology, Massachusetts Institute of Technology. It is equipped with a variable speed belt upon which the samples were carried to the source. A detailed description of the installation and use of the Resonant Transformer has been described by Knowlton, Mahn, and Ranftl (1953).

2. Dosages

The irradiation doses used for both roast beef and ham slices were:

0 rep_{93}
100 x 10^3
200
400
600
800
1000
1500

These doses were obtained by the following operating conditions:

Dose (rep_{93})	Beam Current (milliamps)	Exposure (seconds)	Belt Speed (fps)
100	0.064	20	25
200	0.150	20	25
400	0.115	45	10
600	0.180	45	10
800	0.240	45	10
1000	0.310	45	10
1500	0.460	45	10

(Distance from source to conveyor was 5 inches)

Doses were intermittently checked with manganese glass dosimeters. These were placed on the surface of and beneath a slice and thus measured both the dose supplied to the slice and the dose absorbed by the slice. The dosimeters were held for one hour at room temperature, after which the optical density at 520 mμ in a Beckman Spectrophotometer was measured. The dose is found by calculation or directly from a plotted relationship, and approximated to the nearest 10^3 rep_{93}.

The nature of the distribution of ionization density in the absorbing material when exposed to high energy electrons is important. The absorption is non-uniform, and there is a limiting maximum range. The range with this instrument is 2.8 gm/sq mm. The characteristic ionization density curve of electron absorption shows that 60 per cent of the ionization occurs just beneath the surface, and reaches a maximum at about a third of the range, and rapidly drops to zero. Proctor and Goldblith (1953) investigated the absorption of radiation energy, and showed the importance of sample thickness and the variations found with ionization distribution. They derived an expression to describe the relationship between survivors and D_o values (mean lethal dose, which is the dose required to leave a surviving fraction 1/e or 37 per cent of the initial population) for samples given the same average dose with uniform and non-uniform distribution.

3. Sample Preparation

Samples were prepared, except for packaging, in a manner identical with that described for thermal processing (Section IVc-A).

These samples were packed in Vectrod heat-sealed polyethylene-laminated Mylar, described in Section III-B.

The inoculated Micrococcus pyogenes, var. aureus was an enterotoxin producing strain.

4. Sample Treatment

The samples were irradiated immediately after being packaged. Following irradiation at each dose level, the samples were refrigerated until bacterial counts were made. Plating was begun within 30 minutes after processing. Duplicate samples for freezing were processed, and transferred to the 0°F freezer.

5. Counting of Treated Samples

The slices, weighing 22 gm, were removed from the package by cutting one end and one side of the envelope with scissors that were flamed just before use. Each slice was allowed to slip from the package into a sterile Blendor cup. One hundred ninety-eight ml of sterile, distilled water were added, producing a dilution of 1:10, and the sample was blended at high speed for two minutes. Dilutions were made and plated in duplicate on Tryptone Glucose extract agar. The scissors were washed with hot detergent solution, rinsed, wiped to remove excess water and flamed before further use.

Counts were made in accordance with Standard Methods for the Examination of Dairy Products (1953) using

Tryptone Glucose Extract Agar as the culture medium. Plates were incubated for 48 hours at 37°C.

6. Treatment of Data

Duplicate counts were averaged for each sample. In each run, two non-irradiated slices were used for controls. These were considered to represent 100 per cent survival. In calculating the percent survival the average count obtained with irradiated material was divided by the average count obtained with the unirradiated controls and this fraction was multiplied by 100. The calculated "percent survival" values were plotted as ordinate values and the corresponding irradiation dose as the abscissa values on semi-logarthmic graph paper.

B. RESULTS AND DISCUSSION

Ionizing radiation treatment was carried out to evaluate the application possibilities of this form of processing. Except for the actual processing operation, the conditions were held as similar as possible to those of the heat processing of slices described in Section IVc. The purpose of both methods was to determine the effect of each process on enterotoxic Micrococcus pyogenes, var. aureus inoculated in large numbers into slices of ham and roast beef, and to evaluate the usefulness of each method for commercial practices.

Figures 56 and 57 show the survival curves of Micrococcus pyogenes, var. aureus in roast beef and ham slices. Both curves are similar, and show that the type of meat had no effect on the resistance of Micrococcus pyogenes, to ionizing irradiation.

Both survival curves show two distinct phases, the first between 0 and 200 x 10^3 rep, the second between 200 to 1500 x 10^3 rep. There are two possible explanations for the change of slope obtained in the survival curves. The slices were not sterilized before inoculation, and thus two types of bacterial flora must be considered, the inocu-

lated Micrococcus pyogenes, var. aureus and the resident mixed population. The initial part of the curve probably represents the lethality of most of the inoculum, the later part, the lethality of any residual inoculation, and also, of more importance, the indigenous flora. In this case, the inoculated organism appears to be generally less resistant to radiation than is the original flora.

A second factor could be due to the possibility that the destruction of bacteria by ionizing radiations does not take place as a first order reaction. This would assume that a lag in dose response is first seen, followed by a brief, initially high lethality, which then decreases along a constant curve. However, in the work reported by Proctor and Goldblith (1953), which showed survival curves for a number of organisms exposed to both cathode and gamma rays, variable rates of lethality were not seen with single strains.

The same authors have shown that a sterility dose of 458×10^3 rep is required to reduce a Micrococcus pyogenes, var. aureus population of 16×10^7 to 1 organism per gram. The survival curves show that a transition occurs at approximately 200×10^3 rep. This point, however, cannot be taken as the dose where the inoculated cells are all eliminated, and the destruction of the bacterial flora of the meat has begun. The indigenous flora is being destroyed at lower levels at a given rate, but the lethality of the

inoculated organism, present in much larger numbers, causes this rate to increase sharply.

It is difficult to compare the results of this work with results reported in the literature. Differences in substrate types, physical state of the media, the presence of resident microorganisms, and possible sensitivity variation of specific strains, combine to make an exact comparison impossible.

The non-uniformity of the energy absorption and slice thickness variability further prevent statements of doses in terms of quantitative dose distribution throughout the slices. For this reason, the indicated doses refer only to the radiation received on the surface. These values are descriptive, however, of doses that might be used in a commercial processing situation.

The energy level of 1 M.e.v. is sufficient, in terms of energy input and range of penetration, for the processing of these slices. This is advantageous because less capital investment in apparatus, instrumentation, and installation would be required for this energy level than is needed for higher energy levels. Because of the dimensions of most foods other than slices,higher electron energies are required due to the need of greater penetration. Gamma radiation is used when very high penetrability is required.

There is another advantage in using a slice of the order of the thickness used in this work, for it was thin enough (average thickness of 2.1 mm) to require only a single irradiation treatment. Thicker materials must frequently be irradiated from two sides in an attempt to overcome the lack of penetration. A single treatment is obviously preferred.

Approximately 2.5×10^6 rep were required to reach the level of end point destruction which, in this work, was taken to be 1 survivor in material having an initial count of 10×10^6 organisms (a decrease in numbers of seven logarithmic cycles). At these levels, off flavor and other undesirable organoleptic changes can be a problem, particularly with the uncured beef. Erdman and Watts (1957) carried out work at various radiation levels with the cured meats, ham or bologna. They showed that doses of gamma-radiation up to 2×10^6 rep caused severe color losses, off odors, loss of free sulfhydryl groups and great decreases in nitrite.

Such factors would indicate the inadvisability of setting up an irradiation program to sterilize meat slices of this kind. Levels of about one million rep might be entirely satisfactory for these products if ascorbate were added for protection against oxidative changes, together with seasoning compounds to mask any flavor changes.

The advantages of an irradiation program, however, are many. Processing in the absence of heat has a distinct advantage, the rendering of fat and water from the meat substance is avoided, which was the prime quality defect experienced with heat treatment. Although the conditions of time and temperature used in heat treating slices were not too severe, the use of irradiation would avoid the slightest deleterious changes associated with heat.

There is another advantage provided by the use of ionizing radiations. If it is desirable to combine bread slices and filling before processing into a single package, treatment by irradiation can easily be carried out with the two pieces of bread in intimate contact, and the meat slice placed on top of the bread. This position would enable the meat to be easily irradiated. Such processing would, in fact, be almost identical to the work described in this section with the difference that the meat would be supported by two slices of bread. Cathode rays at one M.e.v. do not usually penetrate beyond the thickness of the meat slice. After processing, the frozen-vacuum operation could be carried out, followed by heat sealing of the package and storing.

Another possibility in the processing of beef slices alone lies in the combination of radiation with another form of processing. The combination with freezing is

described in the next section. Kempe (1955) found that the destruction by heat of Clostridium botulinum was accelerated by pre-irradiation. Sterility values were found to be significantly lowered (four fold) in this work. The effects increased with dose. Kan (1956) found similar results with spores of P.A. No. 3679 and Bacillus cerrus.

The combination of processing methods could result in total sterilization of meat slices. Pasteurization was the goal of this work, but this combination is a potential sterilizing procedure, which might be developed to a level of high bacterial destruction with a minimum of organoleptic changes.

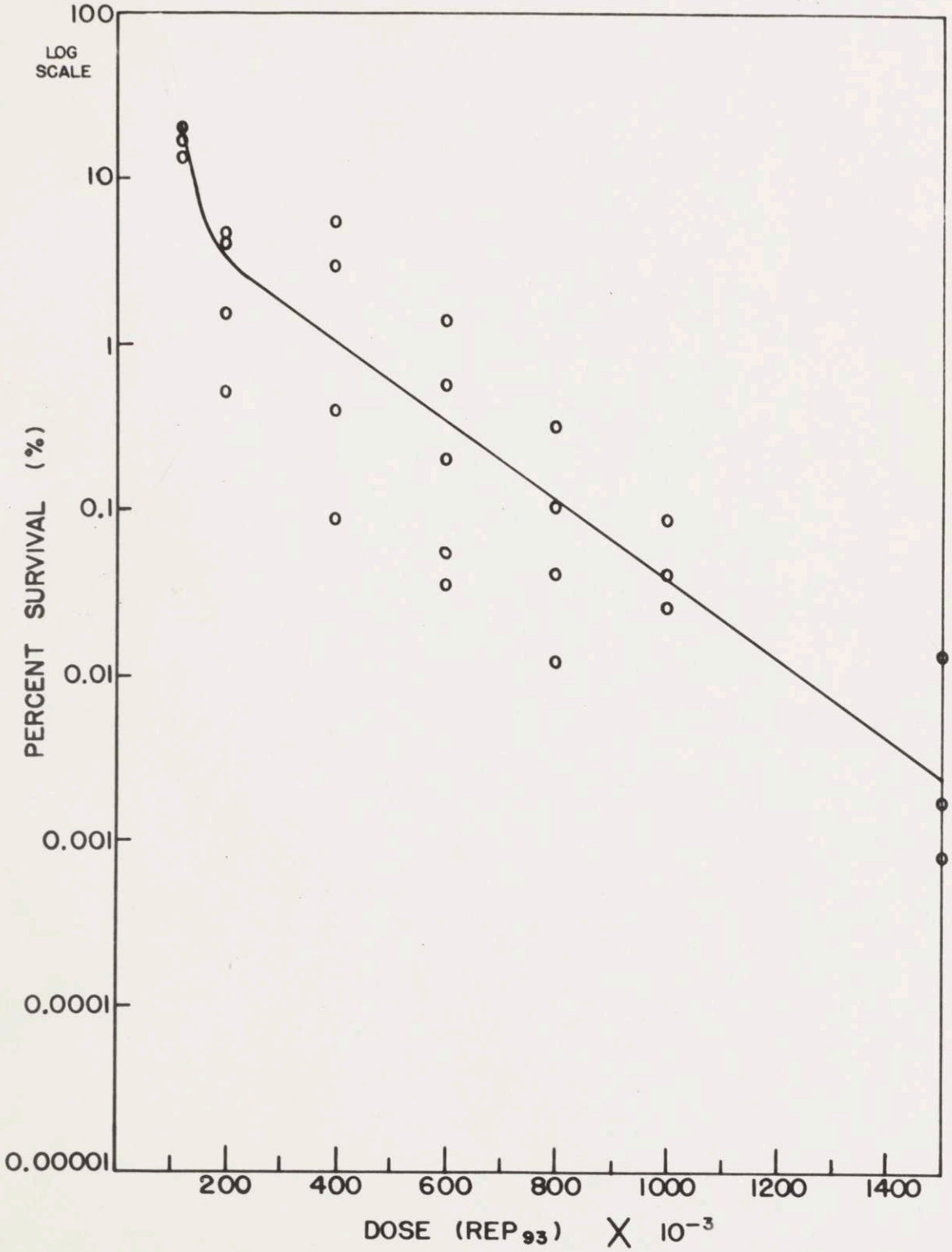

Figure 56. Survival Curve of an Inoculated Enterotoxin Producing Strain of M. pyogenes, var. aureus and Flora in Boiled Ham Slices Irradiated with 1 m.e.v. Cathode Rays.

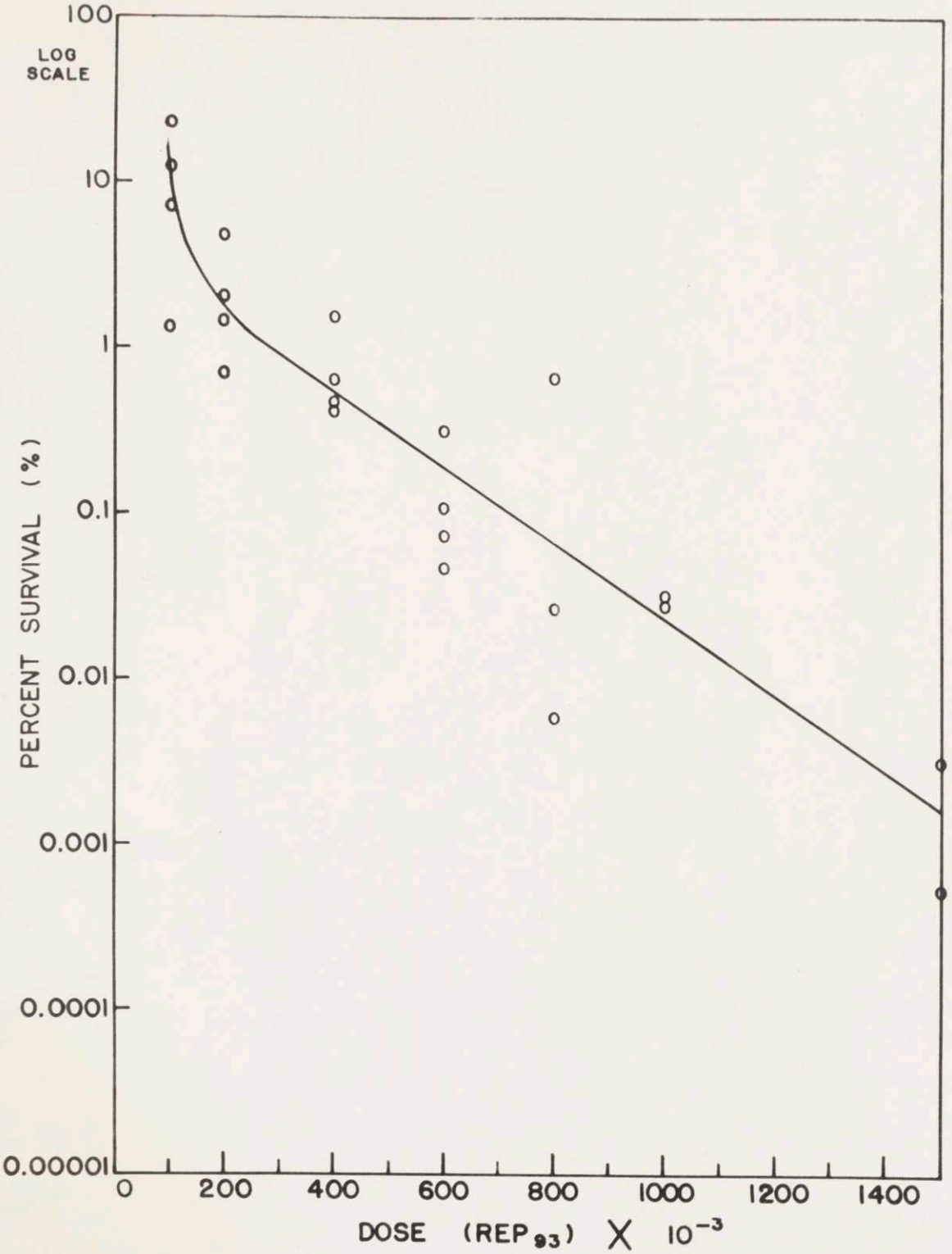

Figure 57. Survival Curve of an Inoculated Enterotoxin Producing Strain of M. pyogenes, var. aureus and Flora in Roast Beef Slices Irradiated with 1 m.e.v. Cathode Rays.

VII.

DESTRUCTION OF MICROCOCCUS PYOGENES, VAR. AUREUS BY FREEZING HAM AND ROAST BEEF SLICES

VII. DESTRUCTION OF MICROCOCCUS PYOGENES, VAR. AUREUS BY FREEZING HAM AND ROAST BEEF SLICES

A. EXPERIMENTAL PROCEDURE

1. Samples

Samples for this work were obtained from slices of boiled ham and roast beef that had been either heated in aluminum envelopes or irradiated in laminated polyethylene-Mylar envelopes. All samples were inoculated with the enterotoxic strain of Micrococcus pyogenes, var. aureus used in this work before being processed.

Prior to freezing, samples were processed under the following conditions:

(a) Heated at 185°F

0 minutes
5 "
10 "
15 "

(b) Irradiated at 1 M.e.v.

0×10^3 rep_{93}
200 "
400 "
800 "
1500 "

2. Procedure

As previously described, the samples were frozen at 0°F immediately after processing. Duplicate samples were plated on Tryptone Glucose Extract Agar immediately after processing, and plates were incubated at 37°C for 48 hours. The frozen samples were held for 24 hours at 0°F before plate counts were made.

Plating was carried out after first blending the sample, weighing 22 gm, in a Waring Blendor with 198 ml of sterile, distilled water after the slice had been removed aseptically from the package. This produced a 1:10 dilution. Samples were not thawed before being blended.

The techniques of counting are described in Section IVc-A.

3. Treatment of Data

Duplicate counts were averaged for each slice, and the counts were expressed on a one gram basis. Calculations of reduction were based on the mean count for the duplicate slice that had not been frozen. Results were expressed as percent reduction and prepared in tabular form.

B. RESULTS AND DISCUSSION

The interest in freezing arises from the necessity to store foods that may be processed by one of the procedures previously developed in this work under frozen conditions. This need is based on the preservation of physical and chemical quality and the suppression of microbial growth.

Although great variability is reported in the literature concerning the lethality of the freezing process, it is generally agreed that there is some reduction in bacterial numbers due to such treatment. This reduction varies with substrates, temperatures, the type of organism and the conditions and times of holding. Because of the common appearance of Micrococci in meat products, the danger of contamination during handling, and the hazardous toxic feature associated with enterotoxin produced by Micrococci, information relevant to to the reduction of these bacteria was of importance.

The substrates used in this work were meat slices previously treated by the simulated commercial operations of heating and irradiation. Homogenates were not used because it is unlikely that homogenates would give results comparable to those obtained with meat slices due

to the vast differences in physical structure, in addition to the fact that homogenates contain as much more water as meat slices. An understanding of the magnitude of the lethality associated with freezing, and an observation of any influence on this lethality of the preprocessing operations, were attempted.

The average results for each processing condition are shown in Table 2. These results show the reduction observed after holding the product for 24 hours at 0°F, and are in units of percent reduction. Three frozen slices were used for the determination of each point.

The mean results for each process and substrate show the lethality to be in a range of 55 to 61 per cent of the initial concentration. Although this is a relatively narrow range, the total variation is from 30 to 86 per cent. There was no difference between results obtained with roast beef or ham slices, nor was any difference shown between particular processing conditions.

These values seem to confirm the consistent reports in the literature of the high resistance of Micrococcus pyogenes, var. aureus to freezing. This reduction, however, is still of value when the total pasteurization concept is considered. It is a safety factor, which can be used to reduce the fractions of microorganisms surviving processing.

These results are in close agreement with those observed by Kiser and Beckwith (1942), who found an average of 59 per cent reduction of a mixed bacterial flora from fish during 15 days of storage at -20°C.

The only other work with frozen sandwich fillings noted by the author was carried out by Geisler (1952) who found that in salad-type fillings, after eight weeks of storage, the mean counts were 10 to 30 per cent of the original values.

Bacterial lethality is assumed to continue during the period of frozen storage. This rate, however, is much slower than the destruction associated with the initial freezing. As slices in this work were held for only 24 hours, a further decrease over that shown might be expected with longer holding time. The purpose in freezing for so short a period of time was to find the effects attributable only to the freezing operation.

TABLE 2

Reduction of Total Counts After Freezing for 24 Hours at 0°F Units of Percent Reduction

Sample Description	Ham	Roast Beef
Thermal Process (185°F)		
0 min	56 %	68
5	47	66
10	56	58
15	76	51
	$\bar{x}$ = 59	$\bar{x}$ = 61
Irradiation (1 M.e.v.)		
0 x 10^3 rep_{93}	64 %	30
200 x 10^3	58	65
400 x 10^3	72	38
800 x 10^3	63	86
1500 x 10^3	42	55
	$\bar{x}$ = 60	$\bar{x}$ = 55

VIII.

SUMMARY AND CONCLUSIONS

VIII. SUMMARY AND CONCLUSIONS

1.

Using Micrococcus pyogenes, var. aureus as the test organism, thermal death times were determined in ham homogenate containing 13 per cent solids. Rates of destruction at 125°, 140°, 155°, 170°, 185°, and 200°F were found by the capillary tube method developed by Stern.

From 125° to 140°F, the slope of the thermal death time curve had a value of 11.3°F. This is in agreement with values reported in the literature. At temperatures above 140°F, however, the resistance of the organism increased. The thermal death time curve, plotted on a semi-logarithmic graph appeared to be linear below 140°F, and curved above this temperature, which was not in agreement with published data. Results found in the literature are generally based on work at lower temperatures, which is then extrapolated through the higher ranges. The results of this investigation show that such extrapolation may not be justified.

2.

A method was developed for the rapid screening of chemical and antibiotic additives for their effect upon the thermal resistance of Micrococcus pyogenes, var. aureus at 140°F. Both ham and roast beef homogenates were used as substrates; lethality was found to be greater in the latter.

The presence of hydroxylamine, allylisothiocyanate, ascorbates alone and in combination with hydroxylamine, and hydroxylamine combinations with propionates all significantly reduced the thermal resistance of the test organism in ham.

With roast beef homogenate, the same chemicals were effective, as well as sorbic acid alone and in combination with hydroxylamine or nitrite, sodium laurate with and without the combination of hydroxylamine, and acetic acid at pH 5.0.

The antibiotics Aureomycin, Terramycin, subtilin and nisin were all effective in reducing the resistance of the organism to heat.

3.

Packaging was developed for purposes of processing meat slices by air oven heat and cathode ray irradiation. A packaging method for dielectric heating was also found, but this method of heating was abandoned because of its

severe effects upon the quality of meat slices.

A method was developed for the packaging of bread slices for frozen storage. Individual slices become stale in air much more rapidly than do whole loaves, and the use of vacuum storage was indicated. A sufficiently high vacuum could not be achieved under normal conditions because the rapid exhaustion of air caused a collapse of crumb cells. To achieve a sufficiently high vacuum, the slices were placed in an unsealed polyethylene-laminated Cryovac or Mylar envelope, and were frozen at 0°F. In the frozen state, the crumb structure was rigid enough to permit the application of high vacuum. The package was heat-sealed, and returned to frozen storage. When thawed, the slice maintained its structure. A considerable extension of storage life was obtained by this procedure.

4.

Boiled ham and roast beef slices, packaged in aluminum foil envelopes, were processed in an air oven at temperatures of 185°, 200°, 250°, and 300°F. These slices, containing a high initial concentration of an enterotoxin producing strain of Micrococcus pyogenes, var. aureus, were processed under conditions simulating

commercial practices. It was possible to reduce the total count to an assumed end point of 99.99999% reduction without causing extensive deterioration of the meat quality.

During heating, Micrococcus pyogenes, var. aureus in ham showed a consistently slightly greater lethal rate than when present in roast beef. There were only slight changes in the lethal rate between temperatures of 250° and 300°F. The destruction rate increased sharply from 185° to 200°F, but then gradually decreased until a temperature of 250°F was reached.

5.

Boiled ham and roast beef slices, inoculated with Micrococcus pyogenes, var. aureus, packaged in polyethylene-laminated Mylar envelopes, were irradiated with cathode rays at doses of 100 to 1500 x 10^3 rep_{93}. There was no difference in bacterial lethality in roast beef and boiled ham slices. In both substrates, survival curves were characterized by two segments. The first was an initially rapid rate of destruction, followed by a lower lethal rate at higher doses. It is likely that the second phase was due to the destruction of resistant bacteria constituting the original flora of the meat.

To arrive at the 99.99999% lethality level achieved by heating, an irradiation dose of approximately 2.5×10^6 rep was required. The chief advantage of irradiation was the reduction of the primary defect of heating, that of rendering fat and moisture from meat slices.

6.

Meat slices processed by heat and radiation were frozen at 0°F for 24 hours to evaluate the effect of the freezing action on the viability of the microorganisms present. There was no difference in bacterial lethality between roast beef and ham, nor did the various conditions of processing influence the extent of lethality due to freezing. Freezing reduced the bacterial population to approximately 40 per cent of the initial count.

IX.

RECOMMENDATIONS FOR FUTURE WORK

IX. RECOMMENDATIONS FOR FUTURE WORK

There are two general directions that might be followed in the development of future work concerning the processing of snack-type foods. These involve a continuation of both fundamental and applications concepts. More understanding of the influence of environmental conditions on the growth and death of microorganisms is important for purposes of increasing the scale of the operation from an experimental basis to one of commercial operations. It is also important to carry out experimental procedures at the pilot plant level for in no other way can unconsidered situations become apparent.

The following is a list of recommendations for the continuation of the work developed in this investigation:

1. A study should be made of the reactions of clostridial spores under conditions similar to those under which the sensitivity of Micrococcus pyogenes, var aureus was determined. This would be the approach to a microbiological evaluation of processes having as their goal

conditions which would provide absolute or "commercial" sterility in these foods.

2.

It may be possible to find a combination of radiation and heating together with freezing and perhaps added chemical and antibiotic substances which will accomplish a state of sterility.

3.

Investigations initiated in the near future should include the application of promising chemical and antibiotic substances as dips for meat slices prior to processing. These materials should be those that altered the heat resistance of Micrococcus pyogenes, var. aureus at 140°F.

4.

The fabrication of a butter-like spread for sandwiches could be carried out. This spread should have several desirable properties. It should protect the filling from air and dessication. A compound, as ascorbate, could be added to the spread to aid further in the chemical protection of the filling and to amplify the lethal effect of heat. Chemical antioxidants might also be added for the prevention of rancidity. It would

be possible to prepare such a material, which could be used either as a conventional spread or, in the liquid state, as a dip for the filling. A butter flavor would probably be desired and could be added, and seasonings such as monosodium glutamate could also be incorporated for accenting and maintaining the flavor of the filling. The fat used in the spread could be made very unreactive with oxygen, and physical properties, as melting point and texture, could be specified.

5.

The application of the processing techniques developed in this work should be attempted with other food products. Sliced turkey, which is now obtainable in boneless rolls, is a logical product for this purpose. Thought should also be given to the use of sandwich filling materials other than those in slice form. Salads could be used which could be processed in patties. The problem of contamination by mayonnaise could probably be eliminated in the processing treatment of the salad.

It is possible that the mayonnaise, or dressing materials used in salads could be fabricated to include a chemical and antioxident material, so that during subsequent processing of the salad, it would aid in the protection of the product against bacteria, as well as

chemical deterioration. The intimate contact of salad dressing with the other ingredients in a salad makes this use quite desirable.

6.

The limits of time and temperature during which pasteurized items can be held prior to consumption without causing possible public health problems should be defined, using as the basis, growth rates of micro-organisms after processing and freezing and the production rates of bacterial enterotoxin. The relationship between the numbers of bacterial cells and the hazard of entero-toxin production should be established. It might be possible to reduce the ability of bacteria to produce enterotoxin by applying a suitable processing scheme. In any event, time is required for a small number of organisms surviving processing to grow and become a potential danger, especially after freezing. If the con-ditions of incubation required in such cases could be determined, it would be of the utmost value.

7.

When having arrived at a processing scheme suitable for a given set of specifications and products, formal organoleptic panel tests should be made on the quality of the products to determine storage limits, optimum pro-

cessing conditions, and methods of extending quality during storage.

8.

Of great interest and ultimate importance in thermal process calculations is the nature of the thermal death response at higher temperatures. This should be further studied with several organisms, and survival data should be gathered over a broad temperature range to evaluate the relationship of the slope of the thermal death time curve with temperature. The accrued information should be treated by both conventional calculation methods and the kinetic method.

9.

Some of the information determined in this research might be applicable to industrial food processing methods. For example, it might be effective to infuse a combination of chemicals, such as ascorbate and hydroxylamine, into whole hams before processing. This might lessen the resistance of the flora to heat processing, and result in a more effective preservative action. Similarly, such methods should be evaluated in comminuted products as frankforts and bologna. Many other application areas are conceivable.

A P P E N D I C E S

A.

TABLES OF SURVIVOR COUNTS OF MICROCOCCUS PYOGENES, VAR. AUREUS, ST. 209, SUSPENDED IN HAM HOMOGENATE AND HEATED IN CAPILLARY TUBES AT TEMPERATURES OF 125° TO 200°F.

HAM HOMOGENATE

125°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.043	160	$1:10^5$	38×10^7	
A2	0	.046	190	10^5	41×10^7	
A3	0	.034	145	10^5	43×10^7	
A4	0	.037	140	10^5	38×10^7	
MEAN					40×10^7	100%
B1	10	0.042	210	$1:10^4$	50×10^6	13%
B2	10	.036	95	10^5	26×10^7	65
B3	10	.035	70	10^4	20×10^7	50
B4	10	.044	95	10^5	22×10^7	55
C1	20	0.052	60	$1:10^5$	12×10^7	30%
C2	20	.039	55	10^5	14×10^7	35
C3	20	.043	280	10^4	65×10^6	16
C4	20	.043	40	10^5	11×10^7	27
D1	30	0.034	200	$1:10^4$	59×10^6	15%
D2	30	.039	190	10^4	49×10^6	12
D3	30	.034	280	10^4	82×10^6	20
D4	30	.049	210	10^4	43×10^6	11

HAM HOMOGENATE

125°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated Plate Count per gram	Percent Survival (%)
A1	0	0.044	280	10^5	64×10^7	
A2	0	.042	240	"	37×10^7	
A3	0	.044	220	"	50×10^7	
A4	0	.038	290	"	76×10^7	
MEAN					63×10^7	100%
B1	5	.044	130	10^5	30×10^7	47
B2	5	.045	230	"	51×10^7	81
B3	5	.041	110	"	27×10^7	43
B4	5	.047	180	"	38×10^7	60
C12	10	.091	240	10^5	26×10^7	41
C34	10	.077	270	"	35×10^7	56
D12	15	.110	Lost	10^5	--	--
D34	15	.102	230	"	23×10^7	37
E13	20	.136	230	10^5	17×10^7	27

HAM HOMOGENATE

140°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.057	55	10^6	94×10^7	
A2	0	.052	45	"	86×10^7	
A3	0	.055	60	"	110×10^7	
A4	0	.056	60	"	105×10^7	
MEAN					100×10^7	100%
B12	1.5	.083	70	10^5	84×10^6	8.4
B34	1.5	.085	60	"	71×10^6	7.1
C12	3.0	.087	150	10^4	17×10^6	1.7
C34	3.0	.080	105	"	13×10^6	1.3
D12	4.5	.075	200	10^3	27×10^5	0.27
D34	4.5	.084	290	"	34×10^5	0.34
E13	6.0	.141	70	10^3	50×10^4	0.050

HAM HOMOGENATE

140°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.058	230	10^5	40×10^7	
A2	0	.060	235	"	39×10^7	
A3	0	.054	200	"	37×10^7	
A4	0	.055	210	"	38×10^7	
MEAN					39×10^7	100%
B12	1.5	.101	110	10^5	110×10^6	35
B34	1.5	.102	45	"	44×10^6	12
C12	3.0	.110	160	10^4	150×10^5	3.8
C34	3.0	.098	120	"	120×10^5	3.1
D12	4.5	.101	80	10^3	79×10^4	0.20
D34	4.5	.099	55	"	54×10^4	0.14
E13	6.0	.176	250	10^2	155×10^3	0.040

HAM HOMOGENATE

155°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.057	35	$1:10^6$	61×10^7	
A2	0	.057	30	10^6	59×10^7	
A3	0	.050	270	10^5	54×10^7	
A4	0	.057	40	10^6	70×10^7	
MEAN					61×10^7	100%
B1	1.0	0.056	250	$1:10^4$	45×10^6	7.4
B2	1.0	.043	110	10^4	26×10^6	4.3
B3	1.0	.042	120	10^4	29×10^6	4.8
B4	1.0	.050	90	10^4	18×10^6	3.0
C12	2.0	0.098	55	$1:10^3$	56×10^4	0.092
C34	2.0	.088	140	10^4	16×10^5	0.26
D12	3.0	0.089	30	$1:10^2$	34×10^3	0.0056
D34	3.0	.105	55	10^1	52×10^2	.00085
E14	4.0	0.174	90	$1:10^1$	51×10^2	0.00085
			155°F			
A1	0	0.043	160	$1:10^5$	38×10^7	
A2	0	.046	190	10^5	41×10^7	
A3	0	.034	145	10^5	43×10^7	
A4	0	.037	140	10^5	38×10^7	
MEAN					40×10^7	100%
B1	2.5	0.038	55	$1:10^2$	15×10^4	0.038
B2	2.5	.036	90	10^2	25×10^4	.063
B3	2.5	.041	80	10^2	20×10^4	.050
B4	2.5	.031	35	10^2	11×10^4	.011

HAM HOMOGENATE

170°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.058	65	$1:10^5$	11×10^7	
A2	0	.039	55	10^5	14×10^7	
A3	0	.041	35	10^5	9×10^7	
A4	0	.053	95	10^5	18×10^7	
MEAN					13×10^7	100%
B1	1.0	0.046	60	$1:10^4$	11×10^6	9.3
B2	1.0	.044	110	10^2	25×10^4	0.18
B3	1.0	.032	210	10^2	66×10^4	0.51
B4	1.0	.032	90	10^3	31×10^5	2.9
C1	1.5	0.044	75	$1:10^2$	17×10^4	0.13
C2	1.5	.035	40	10^3	11×10^5	.85
C3	1.5	.031	95	10^1	31×10^3	.024
C4	1.5	.043	180	10^1	42×10^3	.032
D1	2.0	0.046	250	$1:10^1$	54×10^3	0.042
D2	2.0	.041	35	10^1	85×10^2	.0065
D3	2.0	.032	15	10^1	47×10^2	.0036
D4	2.0	.037	2	10^1	54×10^1	.00042
E14	3.0	0.144	7	1:5	24×10^1	0.00018

HAM HOMOGENATE

170°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.045	60	$1:10^5$	13×10^7	
A2	0	--	--	--	--	
A3	0	.036	65	10^5	18×10^7	
A4	0	.038	20	10^5	18×10^7	
MEAN					16×10^7	100%
B12	1.0	0.061	60	1:5000	49×10^5	3.8
B34	1.0	.073	40	1:5000	27×10^5	1.7
C12	1.5	0.068	80	1:500	59×10^4	0.38
C34	1.5	.076	270	1:50	17×10^3	.011
D14	2.0	0.180	45	1:50	13×10^3	0.0081
E14	2.5	0.147	50	1:5	17×10^2	0.0011

HAM HOMOGENATE

185°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.035	40	$1:10^5$	11×10^7	
A2	0	.048	20	10^5	15×10^7	
A3	0	.037	40	10^5	11×10^7	
A4	0	.043	45	10^5	10×10^7	
MEAN					12×10^7	100%
B1	1.0	0.050	45	$1:10^2$	90×10^3	0.075
B2	1.0	.045	35	10^2	78×10^3	.065
B3	1.0	.039	20	10^1	51×10^2	.0043
B4	1.0	.040	5	10^1	13×10^2	.0010
C1	1.5	0.040	1	1:5	60	.00005
C2	1.5	.041				
C3	1.5	.041	0	1:5	0	0
C4	1.5	.053	13	1:5	12×10^2	.0010

HAM HOMOGENATE

185°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.032	90	$1:10^5$	28×10^7	
A2	0	.035	110	10^5	32×10^7	
A3	0	.040	135	10^5	34×10^7	
A4	0	.036	130	10^5	36×10^7	
MEAN					32×10^7	100%
B1	0.5	0.042	150	$1:10^2$	36×10^4	0.10
B2	0.5	.038	280	10^2	73×10^4	0.23
B3	0.5	.038	270	10^3	71×10^5	2.2
B4	0.5	.034	115	10^3	54×10^5	1.1
C1	1.5	0.037	5	1:5	68×10^1	0.00021
C2,3*	1.5	.072	6	1:5	41×10^1	0.00013
C4	1.5	.031	20	1:50	32×10^3	0.01

* C2 and C3 plated together.

HAM HOMOGENATE

200°F

Sample	Heating Time (min)	Sample Weight (gms)	T.G.E. Sample Count	Dilution	Calculated plate count per gram	Percent Survival (%)
A1	0	0.032	90	$1:10^5$	28×10^7	
A2	0	.035	110	10^5	32×10^7	
A3	0	.040	135	10^5	34×10^7	
A4	0	.036	130	10^5	36×10^7	
MEAN					32×10^7	100%
B1	10 sec	0.037	240	$1:10^2$	65×10^4	0.20
B2	10	.042	175	10^3	42×10^5	1.3
B3	10	.030	140	10^3	47×10^5	1.5
B4	10	.046	110	10^1	24×10^3	0.0075
C1	20 sec	0.029	30	1:5	52×10^2	0.0016
C2	20	.035	100	1:5	14×10^3	0.0044
C3	20	.032	40	1:50	62×10^3	0.019
C4	20	.042	30	1:5	36×10^2	0.0011
A1	--	--	-	--	--	
A2	0	0.034	90	$1:10^5$	26×10^7	
A3	0	.022	50	$1:10^5$	23×10^7	
A4	0	.030	50	$1:10^5$	17×10^7	
MEAN					22×10^7	100%
B1	30 sec	0.031	7	1:5	11×10^2	0.00050
B2	30	.031	2	1:5	32×10^1	0.00015
B3	30	.034	0	1:5	--	--
B4	30	.035	0	1:5	--	--

B.

TABLES OF SURVIVOR COUNTS OF AN ENTEROTOXIN PRODUCING STRAIN OF MICROCOCCUS PYOGENES, VAR. AUREUS ON UNSTERILIZED SLICES OF ROAST BEEF AND BOILED HAM HEATED AT 185° TO 300°F.

1. ROAST BEEF SLICES

Heating Time (minutes)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
	185°F	
0	250×10^5	100%
5	75×10^4	3.0
10	110×10^2	0.44
15	95×10^2	0.038
0	35×10^6	100%
5	140×10^5	40
5	105×10^5	30
10	100×10^3	0.29
10	55×10^4	1.6
15	70×10^2	0.020
15	40×10^2	0.011
0	180×10^5	100%
10	100×10	0.0055
0	125×10^5	100%
2.5	140×10^5	110
7.5	100×10^3	0.80
12.5	80×10^2	0.06
0	65×10^6	100%
5	170×10^5	26
7.5	30×10^5	4.6
10	35×10^4	0.54
12.5	85×10^3	0.13
15	115×10^3	0.18

	200°F	
0	180×10^5	100%
8	80×10^2	0.04
0	80×10^6	100%
5	75×10^4	0.94
10	150×10	0.0019
10	70×10	0.00088
12	12×10	0.00015
12	35×10	0.00044
0	110×10^6	100%
2	70×10^6	65
2	55×10^6	50
4	100×10^5	9.1
4	270×10^4	2.4
6	225×10^2	0.020
6	45×10^4	0.41
0	65×10^6	100%
2	50×10^6	77
4	230×10^4	3.5
6	45×10^4	0.69
8	60×10^2	0.0092
10	30×10^2	0.0046
0	290×10^5	100%
4	50×10^4	1.7
6	260×10^3	0.90
8	60×10^2	0.021
10	90×10	0.0031

	250°F	
0	95×10^5	100%
1	90×10^5	95
2	30×10^4	3.2
3	95×10^2	0.1
4	7×10	0.00073
5	5×10	0.00005
0	35×10^6	100%
1	240×10^5	69
2	110×10^3	0.31
3	65×10^2	0.018
4	30×10^2	0.0086
	300°F	
0	180×10^5	100%
2	20×10	0.0011
4	10×10	0.00055
0	200×10^5	100%
1	160×10^5	80
2	40×10^3	0.20
3	30×10^2	0.015
4	15×10	0.00075
0	65×10^6	100%
1	50×10^5	7.7
2	95×10^3	0.15
3	140×10	0.0022

2. HAM SLICES

	185°F	
0	135×10^6	100%
5	65×10^6	48
10	60×10^4	0.45
15	60×10^2	0.0045
0	240×10^5	100%
5	70×10^5	29
15	65×10	0.0027
0	65×10^6	100%
5	280×10^5	43
5	160×10^5	25
10	80×10^2	0.012
10	195×10^3	0.30
15	35×10^2	0.0054
15	130×10	0.0020
0	140×10^5	100%
5	100×10^5	71
10	30×10^2	0.021
15	12×10	0.00086
0	180×10^5	100%
2.5	170×10^5	95
7.5	110×10^4	6.1
10	45×10^2	0.025

	200°F	
0	35×10^6	100%
6	35×10^4	1.0
8	30×10	0.00086
10	12×10	0.00030
0	180×10^6	100%
2	190×10^5	11
2	40×10^6	22
4	150×10^3	0.083
4	160×10^4	0.89
6	250×10^2	0.014
6	65×10^3	0.036
0	50×10^6	100%
2	120×10^4	2.4
4	135×10^3	0.27
6	270×10^2	0.054
8	20×10	0.00040
0	280×10^5	100%
2	90×10^5	32
4	40×10^4	1.4
6	225×10	0.0080
8	5×10	0.00018
	250°F	
0	250×10^5	100%
1	200×10^5	80
2	240×10^2	0.096
3	105×10^2	0.042
4	30×10	0.0012

	250°F	
0	260×10^5	100%
1	35×10^6	140
2	60×10^5	23
3	70×10	0.0027
0	50×10^6	100%
2	120×10^3	0.24
3	270×10	0.0055
4	40×10	0.0008

	300°F	
0	140×10^5	100%
2	110×10^2	0.076
4	2×10	0.00014
0	30×10^6	100%
1	65×10^5	22
2	110×10^3	0.35
3	40×10	0.0013
4	10×10	0.00033
0	50×10^6	100%
1	140×10^4	2.8
2	220×10^2	0.044
3	240×10^1	0.0048

C.

TABLES OF SURVIVOR COUNTS OF AN ENTEROTOXIN PRODUCING STRAIN OF MICROCOCCUS PYOGENES, VAR. AUREUS SUSPENDED IN 2.5 ML OF ROAST BEEF AND HAM HOMOGENATES, AND HEATED FOR 0 TO 30 MINUTES AT 140°F.

ROAST BEEF HOMOGENATE

Heating Time (min)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
0*	200×10^6	100%
5	65×10^6	33
5	50×10^6	25
5	260×10^6	13
10	35×10^5	1.8
10	45×10^5	2.3
10	100×10^5	5.0
15	90×10^4	0.45
15	270×10^4	1.4
15	170×10^4	0.85
20	280×10^3	0.74
20	180×10^3	0.09
20	70×10^4	0.35
25	190×10^3	0.095
25	110×10^3	0.055
25	165×10^2	0.0083
30	90×10^3	0.045
30	70×10^2	0.0035
30	65×10^3	0.033

* Mean Count of Two Tubes.

HAM HOMOGENATE

Heating Time (min)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
0*	120×10^6	100
5	125×10^5	10
5	35×10^6	29
5	180×10^5	15
10	120×10^4	1.0
10	30×10^5	2.5
10	35×10^5	2.9
15	180×10^3	0.15
15	115×10^4	0.96
15	75×10^3	0.063
20	115×10^3	0.096
20	40×10^3	0.033
20	35×10^4	0.29
25	180×10^3	0.15
25	70×10^3	0.058
25	60×10^3	0.050
30	45×10^2	0.0039
30	280×10^2	0.023
30	40×10^2	0.0033

* Mean Count of Two Tubes.

D.

TABLES OF SURVIVOR COUNTS OF MICROCOCCUS PYOGENES, VAR. AUREUS, F.D.A. 209 SUSPENDED IN 2.5 ML OF ROAST BEEF AND HAM HOMOGENATES HEATED AT 140°F. CHEMICAL AND ANTIBIOTIC SUBSTANCES ADDED TO HOMOGENATES

1. CHEMICAL ADDITION TO ROAST BEEF HOMOGENATE

Heating Time (minutes)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
Control (no chemical addition)		
0*	160×10^6	100%
5	110×10^6	69
10	30×10^6	19
15	175×10^4	1.1
20	50×10^4	0.31
25	50×10^3	0.031
30	125×10^2	0.0078
0*	155×10^6	100%
7.5	150×10^5	10
15	40×10^5	2.7
20	70×10^4	0.47
25	65×10^3	0.043
30	100×10^2	0.0067
0*	150×10^6	100%
30	95×10^2	0.0063
0*	140×10^6	100%
5	50×10^6	36
10	110×10^5	7.9
15	30×10^4	0.21
20	150×10^3	0.11
25	250×10^2	0.018
30	100×10^2	0.0071
0*	155×10^6	100%
10	250×10^4	1.6
15	125×10^4	0.80
20	50×10^3	0.032

* Mean count of duplicate rates of two tubes

0*	175×10^6	100%
20	60×10^3	0.034
25	200×10^2	0.011
0*	50×10^6	100%
25	300×10^2	0.060
30	50×10^2	0.010
0*	200×10^6	100%
10	100×10^5	5.0
20	85×10^3	0.043
30	65×10^2	0.0033
0*	200×10^6	100%
25	100×10^3	0.050
0*	90×10^6	100%
5	75×10^5	8.3
10	200×10^3	0.22
15	30×10^3	0.033
20	45×10^3	0.050
25	95×10^2	0.011
30	220×10	0.0024

HYDROXLAMINE (200 ppm)

0*	150×10^6	100%
5	150×10^5	10
10	30×10^4	0.20
15	70×10^3	0.047
20	105×10^2	0.0070
25	160×10^1	0.0011
30	35×10^1	0.00023

0*	155×10^6	100%
5	135×10^5	8.7
7.5	50×10^4	0.32
10	70×10^3	0.045
15	35×10^3	0.023
0*	275×10^6	100%
5	110×10^3	0.040
10	45×10^3	0.016
15	160×10^2	0.0058
20	140×10^2	0.0051
25	120×10^2	0.0044
0*	210×10^6	100%
5	55×10^6	26
10	40×10^3	0.019
15	180×10	0.00086
20	150×10	0.00072
25	60×10	0.00029
30	5×10	0.000024

HYDROXYLAMINE (100 ppm)

0*	180×10^6	100%
5	110×10^6	61
10	80×10^4	0.44
15	40×10^3	0.022
20	160×10^2	0.0089
25	35×10^2	0.0019
30	165×10	0.00092
0*	85×10^6	100%
5	180×10^4	2.1
10	30×10^3	0.035
15	55×10	0.0065
20	35×10	0.00041
25	30×10^2	0.0035
30	200×10	0.0024

ALLYLISOTHIOCYANATE (200 ppm)

0*	190×10^6	100%
5	60×10^5	3.2
10	115×10^3	0.061
15	150×10^2	0.0079
20	230×10	0.0012
25	130×10^2	0.0068
30	250×10	0.0013
0*	130×10^6	100%
5	120×10^5	9.2
10	150×10^3	0.12
15	30×10^3	0.023
20	110×10^2	0.0085
25	90×10	0.00069
30	25×10	0.00019

SODIUM NITRITE (200 ppm)

0*	240×10^6	100%
5	110×10^6	46
10	150×10^4	0.62
15	170×10^2	0.0071
20	100×10^3	0.042
25	80×10^2	0.0033
30	50×10	0.00021
0*	65×10^6	100%
5	195×10^5	30
10	40×10^4	0.62
15	100×10^2	0.015
20	60×10^2	0.0092
25	55×10	0.00085

0*	170×10^6	100%
5	200×10^5	12
10	35×10^4	0.21
15	130×10^3	0.077
20	270×10	0.0016
25	50×10	0.0029
0*	30×10^6	100%
20	40×10^3	0.13
25	70×10^2	0.023
30	155×10	0.0052

SODIUM NITRITE (200 ppm) and
HYDROXYLAMINE (200 ppm)

0*	160×10^6	100%
5	145×10^5	9.1
10	160×10^3	0.10
15	65×10^2	0.0041
20	55×10^2	0.0034
25	200×10	0.0013
30	5×10	0.000031
0*	180×10^6	100%
5	55×10^4	0.31
10	190×10^2	0.011
15	140×10^2	0.0078
20	170×10	0.00095
25	35×10	0.00019
30	30×10	0.00017

CALCIUM CHLORIDE (0.10%)

0*	85×10^6	100%
5	120×10^5	14
10	150×10^3	0.18
15	100×10^2	0.012

20	120×10^2	0.014
25	70×10^2	0.0082
30	120×10	0.0014
0*	265×10^6	100%
5	90×10^6	34
10	95×10^4	0.36
15	250×10^3	0.095
20	110×10^3	0.041
25	40×10^3	0.017

MAGNESIUM CHLORIDE (0.1%)

0*	265×10^6	100%
5	110×10^6	42
10	160×10^4	0.60
15	80×10^3	0.030
20	75×10^3	0.028
25	30×10^2	0.0012
0*	130×10^6	100%
5	140×10^5	11
10	30×10^5	2.3
15	40×10^4	0.31
20	200×10^2	0.015
25	105×10^2	0.0081
30	120×10	0.00092

ACETIC ACID AT pH 5.0*

0*	190×10^6	100%
5	35×10^5	1.9
10	100×10^3	0.053
15	120×10^2	0.0063
20	30×10^2	0.0015
25	135×10	0.00071
30	40×10	0.000021

* Homogenate was at pH of 5.9 before acid addition.

0*	180×10^6	100%
5	155×10^5	8.6
10	40×10^4	0.22
15	30×10^2	0.0017
20	45×10^2	0.0025
25	55×10	0.00031
30	25×10	0.00015

SODIUM BENZOATE (0.10%)

0*	200×10^6	100%
5	100×10^6	50
10	240×10^5	1.2
15	35×10^4	0.18
20	135×10^3	0.068
25	150×10^2	0.0075
0*	200×10^6	100%
5	70×10^6	35
10	125×10^4	0.63
15	210×10^3	0.10
20	90×10^2	0.0045
25	40×10^2	0.0020
30	95×10	0.00048

SODIUM LAURATE (0.075%)

0*	130×10^6	100%
5	35×10^6	27
10	190×10^3	0.15
15	40×10^3	0.031
20	65×10^2	0.0050
25	260×10	0.0020
30	35×10^2	0.0027

0*	200×10^6	100%
5	30×10^6	15
10	50×10^4	0.25
15	260×10^2	0.013
20	40×10^3	0.020
25	90×10^2	0.0045
30	140×10^2	0.0070

SODIUM LAURATE (0.125%0

0*	130×10^6	100%
5	85×10^5	6.5
10	95×10^3	0.073
15	250×10^2	0.019
20	40×10^2	0.0031
25	120×10	0.00093
30	45×10	0.00035
0*	160×10^6	100%
5	30×10^6	19
10	55×10^3	0.034
15	70×10^2	0.0044
20	60×10^2	0.0038
25	185×10	0.0012
30	150×10	0.00094

SODIUM LAURATE (0.075%) and
HYDROXYLAMINE (200 ppm)

0*	200×10^6	100%
5	190×10^5	9.5
10	80×10^3	0.040
15	70×10^2	0.0035
20	35×10^2	0.0018
25	180×10	0.00090
30	15×10	0.000075

0*	160×10^6	100%
5	50×10^6	50
15	35×10^3	0.053
20	140×10^2	0.010
25	35×10^2	0.0015
30	20×10	0.0014

SODIUM LAURATE (0.075%) and

SODIUM NITRITE (200 ppm)

0*	160×10^6	100%
5	80×10^6	50
10	85×10^3	0.053
15	160×10^2	0.010
20	240×10	0.0015
25	145×10^2	0.0091
30	230×10	0.0014
0*	200×10^6	100%
5	35×10^6	18
10	260×10^3	0.13
15	120×10^3	0.06
20	165×10^2	0.0083
25	240×10	0.0012
30	145×10	0.000073

SORBIC ACID (0.075%)

0*	175×10^6	100%
5	45×10^6	26
10	55×10^4	0.31
15	35×10^3	0.020
20	120×10^2	0.0069
25	90×10^2	0.0051
30	40×10^2	0.0022

0*	235×10^6	100%
5	95×10^6	40
10	145×10^4	0.62
15	200×10^3	0.085
20	55×10^3	0.023
25	230×10^2	0.0098
30	210×10	0.00089
0*	155×10^6	100%
5	55×10^6	35
10	200×10^3	0.13
15	70×10^3	0.045
20	100×10^3	0.065
25	210×10^3	0.14
30	55×10^2	0.0035

SORBIC ACID (0.125%)

0*	155×10^6	100%
5	100×10^5	6.5
10	115×10^3	0.074
15	55×10^2	0.0035
20	140×10^2	0.0090
25	150×10	0.00097
30	70×10	0.00045
0*	50×10^6	100%
5	45×10^5	9.0
10	250×10^2	0.050
15	55×10^2	0.011
20	130×10^2	0.026
25	3×10	0.00006
30	0×10	---

0*	200×10^6	100%
5	55×10^6	28
10	30×10^4	0.15
15	75×10^3	0.038
20	35×10^3	0.018
25	165×10^2	0.0083

SORBIC ACID (0.075%) and
HYDROXYLAMINE (200 ppm)

0*	235×10^6	100%
5	105×10^6	45
10	60×10^4	0.25
15	90×10^3	0.038
20	130×10	0.00055
25	210×10^2	0.0089
30	110×10	0.00047
0*	100×10^6	100%
5	190×10^5	19
10	260×10^2	0.026
15	55×10^2	0.0055
20	95×10	0.00095
25	80×10	0.00080
30	240×10	0.0024
0*	175×10^6	100%
5	95×10^5	5.4
10	30×10^2	0.0017
15	165×10	0.00094
20	70×10^2	0.0040
25	200×10	0.0011
30	110×10	0.00063

SORBIC ACID (0.075%) and
SODIUM NITRITE (200 ppm)

0*	155×10^6	100%
5	250×10^5	16
10	95×10^3	0.061
15	240×10	0.0015
20	45×10^2	0.0029
25	60×10	0.00039
0*	175×10^6	100%
5	35×10^6	20
10	125×10^2	0.0072
15	130×10^2	0.0074
20	290×10	0.0017
25	85×10^2	0.0049
30	40×10	0.00023
0*	100×10^6	100%
5	95×10^5	9.5
10	30×10^3	0.030
15	220×10	0.0022
20	40×10^2	0.0040
25	80×10	0.00080
30	70×10	0.00070

CALCIUM PROPIONATE (0.075%)

0*	175×10^6	100%
5	85×10^6	49
10	85×10^5	4.9
15	105×10^4	0.60
20	60×10^3	0.034
25	110×10	0.00063
30	50×10	0.00029

0*	275×10^6	100%
10	190×10^3	0.069
15	120×10^3	0.044
20	200×10^2	0.0073
25	55×10^2	0.0020
0*	160×10^6	100%
5	90×10^6	59
10	150×10^4	0.94
15	210×10^3	0.13
20	270×10^2	0.017
25	65×10^2	0.0041
30	35×10^2	0.0022

CALCIUM PROPIONATE (0.125%)

0*	175×10^6	100%
5	80×10^6	46
10	55×10^4	0.31
15	170×10^3	0.097
20	250×10	0.0014
25	100×10	0.00057
30	15×10	0.000086
0*	275×10^6	100%
10	45×10^4	0.16
15	65×10^3	0.024
20	140×10^3	0.051
25	200×10^2	0.0073
0*	160×10^6	100%
5	55×10^6	34
10	50×10^3	0.031
15	75×10^3	0.047
20	30×10^3	0.019
25	35×10^2	0.0022
30	250×10	0.0016

0*	150×10^6	100%
5	50×10^6	33
10	95×10^3	0.063
15	100×10^2	0.0067
20	40×10^2	0.0026
25	95×10^2	0.0063
30	60×10	0.00040

CALCIUM PROPIONATE (0.075%) and
HYDROXYLAMINE (200 ppm)

0*	160×10^6	100%
5	90×10^6	56
10	130×10^3	0.081
15	170×10	0.0011
20	100×10	0.00063
25	35×10	0.00022
30	15×10	0.000094
0*	150×10^6	100%
5	60×10^6	40
10	290×10^3	0.19
15	45×10^2	0.0030
20	50×10	0.00033
25	10×10	0.000067
30	40×10	0.00027

CALCIUM PROPIONATE (0.075%) and
SODIUM NITRITE (200 ppm)

0*	150×10^6	100%
5	70×10^6	47
10	45×10^4	0.30
15	250×10	0.0017
20	95×10	0.00063
25	10×10	0.000067
30	25×10	0.00017

0*	160×10^6	100%
5	90×10^6	56
10	35×10^4	0.22
15	140×10^2	0.0088
20	155×10	0.00097
25	65×10	0.00041
30	35×10	0.00022

n-PROPIONIC ACID (0.075%)

0*	175×10^6	100%
5	50×10^6	29
10	100×10^3	0.057
15	240×10	0.0014
20	200×10	0.0011
25	40×10^2	0.0023
30	35×10	0.00020
0*	135×10^6	100%
5	155×10^4	1.1
10	75×10^3	0.055
15	95×10^2	0.0070
20	70×10	0.00052
25	20×10	0.00015
30	110×10^2	0.0088
0*	220×10^6	100%
5	140×10^5	6.3
10	160×10^3	0.073
15	80×10^2	0.0036
20	145×10^2	0.0066
25	30×10^2	0.0014
30	40×10	0.00018

n-PROPIONIC ACID (0.125%)

0*	135×10^6	100%
5	125×10^4	0.93
10	135×10^2	0.010
15	155×10	0.0011
20	145×10	0.0011
25	20×10	0.00015
30	10×10	0.000074
0*	175×10^6	100%
5	115×10^5	6.6
10	60×10^3	0.034
15	30×10^2	0.0017
20	80×10^2	0.0046
25	30×10	0.00017
30	20×10	0.00011

n-PROPIONIC ACID (0.075%) and
HYDROXYLAMINE (200 ppm)

0*	220×10^6	100%
5	70×10^4	0.32
10	110×10^3	0.050
15	155×10^3	0.070
20	75×10^2	0.0034
25	200×10	0.00091
30	50×10	0.00023
0*	135×10^6	100%
5	140×10^4	1.0
10	115×10^2	0.0085
15	85×10^2	0.0063
20	110×10	0.00082
25	20×10	0.00015
30	10×10	0.000074

n-PROPIONIC ACID (0.075%) and
SODIUM NITRITE (200 ppm)

0*	220×10^6	100%
5	45×10^5	2.0
10	50×10^3	0.023
15	110×10^2	0.0056
20	170×10	0.00077
25	240×10	0.0011
30	105×10	0.00048
0*	135×10^6	100%
5	125×10^4	0.93
10	135×10^2	0.010
15	55×10^2	0.0041
20	50×10	0.00038
25	25×10	0.00019
30	30×10	0.00022
0*	175×10^6	100%
5	100×10^5	5.7
10	95×10^3	0.054
15	115×10^2	0.0066
20	60×10^2	0.0034
25	250×10	0.0014
30	20×10	0.00011

L-ASCORBIC ACID (0.10%)

0*	90×10^6	100%
5	45×10^5	5.0
10	55×10^3	0.061
15	80×10	0.00089
20	50×10	0.00056
25	90×10	0.0010
30	85×10	0.00094

0*	240×10^6	100%
5	95×10^6	40
10	150×10^4	0.62
15	120×10^2	0.0050
20	85×10^2	0.0035
25	275×10	0.0011
30	0×10	--
0*	65×10^6	100%
5	230×10^5	35
10	105×10^4	1.6
15	150×10^2	0.023
20	70×10^2	0.011
25	130×10	0.0020
0*	170×10^6	100%
5	135×10^5	7.9
10	55×10^4	0.32
15	150×10^2	0.0088
20	50×10^2	0.0029
25	120×10	0.00071
0*	30×10^6	100%
20	50×10^3	0.17
25	75×10^2	0.025
30	215×10	0.0072

L-ASCORBIC ACID (0.10%) and
HYDROXYLAMINE (200 ppm)

0*	90×10^6	100%
5	115×10^4	1.3
10	45×10^2	0.0050
15	80×10^2	0.0089
20	60×10^2	0.0067
25	125×10	0.0014
30	2×10	0.000022

0*	100×10^6	100%
5	90×10^5	9.0
5	60×10^5	6.0
10	110×10^3	0.11
10	115×10^2	0.015
15	55×10	0.00055
15	35×10	0.00035
20	10×10	0.00010
20	5×10	0.000058
25	2×10	0.000020
25	5×10	0.000058
30	0×10	--
30	0×10	--

L-ASCORBIC ACID (0.10%) and
SODIUM NITRITE (200 ppm)

0*	30×10^6	100%
5	200×10^4	6.7
10	30×10^3	0.10
15	100×10^2	0.033
20	55×10	0.0018
25	15×10	0.00050
30	2×10	0.000067
0*	220×10^6	100%
5	45×10^6	20
10	135×10^4	0.61
15	50×10^2	0.0023
20	145×10	0.00066
25	12×10	0.000055
30	5×10	0.000023

SODIUM ASCORBATE (0.10%)

0*	130×10^6	100%
5	45×10^6	35
10	180×10^3	0.14
15	40×10^2	0.0031
20	30×10	0.00023
25	40×10	0.00031
30	15×10	0.00012
0*	210×10^6	100%
5	120×10^5	5.7
10	60×10^3	0.029
15	50×10^2	0.0024
20	30×10	0.00014
25	15×10	0.000072
30	2×10	0.0000095
0*	160×10^6	100%
5	200×10^5	12
10	250×10^3	0.16
15	145×10^2	0.0094
20	150×10	0.00094
25	13×10	0.000081
30	1×10	---
0*	85×10^6	100%
5	40×10^5	4.7
10	240×10^2	0.028
15	100×10	0.0012
20	250×10	0.0029
25	20×10	0.00024
30	60×10	0.00071

0*	220×10^6	100%
5	110×10^5	5.0
10	140×10^3	0.064
15	195×10	0.00089
20	120×10	0.00055
25	245×10	0.0011
30	205×10	0.00093

SODIUM ASCORBATE (0.10%) and
HYDROXYLAMINE (200 ppm)

0*	210×10^6	100%
5	40×10^6	19
10	250×10^2	0.012
15	65×10	0.00031
20	80×10^2	0.0038
25	1×10	--
30	0×10	--
0*	130×10^6	100%
5	40×10^6	31
10	75×10^3	0.058
15	180×10	0.0014
20	45×10	0.00035
25	20×10	0.00015
30	3×10	0.000023

SODIUM ASCORBATE (0.10%) and
SODIUM NITRITE (200 ppm)

0*	130×10^6	100%
5	30×10^6	23
10	135×10^3	0.10
15	50×10^2	0.0039
20	35×10	0.00027
25	15×10	0.00012
30	10×10	0.000077

0*	210×10^6	100%
5	45×10^6	21
10	125×10^4	0.60
20	145×10	0.00068
25	10×10	0.000048
30	5×10	0.000024

2. CHEMICAL ADDITION TO HAM HOMOGENATE

Heating Time (minutes)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
Control (no chemical addition)		
0*	200×10^6	100%
5	55×10^6	28
10	150×10^5	7.5
15	45×10^4	0.23
20	250×10^3	0.13
25	125×10^3	0.065
30	150×10^2	0.0080
0*	215×10^6	100%
5	100×10^6	47
10	35×10^5	1.6
15	30×10^4	0.14
20	55×10^3	0.036
25	90×10^2	0.0042
30	40×10^2	0.0019
0*	265×10^6	100%
10	230×10^5	8.7
0*	130×10^6	100%
5	65×10^6	50
10	85×10^5	6.5
15	260×10^4	2.0
20	40×10^4	0.31
25	50×10^3	0.030
30	270×10^2	0.021

* Mean of duplicate plate count of two tubes.

0*	60×10^6	100%
5	165×10^5	27
10	110×10^4	1.8
15	30×10^4	0.50
20	130×10^3	0.21
25	35×10^3	0.058
0*	290×10^6	100%
30	95×10^3	0.033
0*	100×10^6	100%
30	60×10^2	0.0060
0*	120×10^6	100%
20	205×10^3	0.17
30	85×10^2	0.0071
0*	135×10^6	100%
15	70×10^4	0.52
30	50×10^3	0.037

HYDROXYLAMINE (0.02%)

0*	50×10^7	100%
5	190×10^6	38
10	170×10^4	0.34
15	50×10^3	0.010
20	90×10^2	0.0018
25	45×10^2	0.00090
30	40×10	0.000080
0*	130×10^6	100%
5	45×10^6	35
10	35×10^5	2.7
15	70×10^4	0.54
20	75×10^3	0.058
25	35×10^2	0.0027
30	105×10	0.00080

0*	100×10^6	100%
30	290×10	0.0029
0*	180×10^6	100%
5	265×10^5	15
10	190×10^4	1.1
15	225×10^3	0.13
20	60×10^3	0.033
25	105×10^2	0.0058
30	90×10	0.00050
0*	250×10^6	100%
10	220×10^4	0.88
15	275×10^3	0.11
20	90×10^3	0.036
25	40×10^2	0.0016
0*	120×10^6	100%
20	200×10^2	0.017
30	50×10^2	0.0042

VITAMIN K_5 (4-Amino-2-Methyl-1-Napthol·HCl) (10 ppm)

0*	135×10^6	100%
5	60×10^6	44
10	55×10^5	4.1
15	85×10^4	0.63
20	30×10^4	0.22
25	115×10^2	0.0078
30	35×10^3	0.026
0*	225×10^6	100%
5	130×10^6	58
10	205×10^5	9.1
15	215×10^4	0.95
20	35×10^4	0.16
25	115×10^3	0.051
30	60×10^3	0.028

VITAMIN K_5 (100 ppm)

0*	130×10^6	100%
5	95×10^5	7.3
10	80×10^4	0.61
15	35×10^4	0.27
20	125×10^3	0.096
25	130×10^2	0.010
30	45×10^2	0.0035
0*	120×10^6	100%
5	200×10^5	17
10	100×10^4	0.83
15	125×10^3	0.10
20	210×10^2	0.018
25	235×10^2	0.020
30	65×10^2	0.0054

MAGNESIUM CHLORIDE (0.10%)

0*	200×10^6	100%
5	90×10^6	45
10	50×10^5	2.5
15	120×10^4	0.60
20	150×10^3	0.075
25	255×10^2	0.013
30	105×10^2	0.0052
0*	125×10^6	100%
5	35×10^6	28
10	75×10^4	0.60
15	50×10^4	0.40
20	125×10^3	0.10
25	255×10^2	0.020
30	35×10^3	0.028

SODIUM SULFITE (0.075%)

0*	200×10^6	100%
5	105×10^6	53
10	45×10^5	2.2
15	180×10^3	0.090
20	35×10^4	0.18
25	135×10^2	0.0068
30	30×10^3	0.015
0*	150×10^6	100%
5	50×10^6	33
10	130×10^5	8.7
15	185×10^4	1.2
20	45×10^3	0.030
25	220×10	0.0015
30	65×10^2	0.0043

DIMETHYLDIHYDRORESORCINOL (0.075%)

0*	115×10^6	100%
5	50×10^6	43
10	190×10^5	17
15	160×10^4	1.4
20	60×10^4	0.52
25	50×10^3	0.044
30	290×10^2	0.025
0*	150×10^6	100%
5	50×10^6	33
10	90×10^5	6.0
15	80×10^4	0.53
20	250×10^3	0.17
25	195×10^2	0.013
30	125×10^2	0.0083

ALLYLISOTHIOCYANATE (200 ppm)

0*	265×10^6	100%
5	70×10^6	26
10	165×10^4	0.62
15	220×10^3	0.083
20	40×10^2	0.0015
25	225×10	0.00085
30	105×10	0.00040
0*	150×10^6	100%
5	30×10^6	20
10	185×10^4	1.2
15	150×10^3	0.10
20	165×10^3	0.11
25	280×10^2	0.019
30	135×10	0.00090
0*	290×10^6	100%
30	165×10	0.00057
0*	150×10^6	100%
5	210×10^5	14
10	55×10^5	3.7
15	85×10^3	0.057
20	180×10	0.0012
25	245×10	0.0016
30	30×10^2	0.0020
0*	190×10^6	100%
5	170×10^5	9.0
10	145×10^4	0.76
15	45×10^3	0.024
20	250×10^2	0.013
25	55×10^2	0.0029
30	120×10	0.00063

ALLYLISOTHIOCYANATE (200 ppm) and
HYDROXYLAMINE (200 ppm)

0*	190×10^6	100%
5	125×10^5	6.6
10	140×10^4	0.73
15	135×10^3	0.071
20	155×10^2	0.0082
25	35×10^2	0.0018
30	150×10	0.00079
0*	210×10^6	100%
5	30×10^6	14
10	55×10^5	2.6
15	55×10^3	0.026
20	40×10^2	0.0019
25	190×10	0.00091
30	180×10	0.00087

BUTYLATED HYDROXTOLUENE (100 ppm)

0*	115×10^6	100%
5	45×10^6	39
10	85×10^4	0.75
15	180×10^3	0.16
20	110×10^3	0.096
25	170×10^2	0.015
30	260×10^2	0.023
0*	120×10^6	100%
5	170×10^5	14
10	230×10^4	1.9
15	50×10^4	0.42
20	55×10^3	0.046
25	30×10^3	0.025
30	210×10	0.0018

BUTYLATED HYDROXYTOLUENE (100 ppm) and
HYDROXYLAMINE (200 ppm)

0*	120×10^6	100%
5	260×10^5	22
10	95×10^4	0.79
15	180×10^3	0.15
20	115×10	0.00096
25	100×10	0.00084
30	80×10	0.00067
0*	115×10^6	100%
5	120×10^5	10
10	35×10^4	0.30
15	80×10^2	0.0070
20	95×10^2	0.0083
25	150×10^2	0.013
30	50×10	0.0043

ACETIC ACID AT pH 6.0

0*	130×10^6	100%
5	60×10^6	46
10	180×10^4	1.4
15	60×10^4	0.46
20	140×10^3	0.11
25	90×10^2	0.0069
30	135×10^2	0.010
0*	290×10^6	100%
5	45×10^6	16
10	210×10^3	0.073
15	70×10^4	0.24
20	170×10^3	0.059
25	100×10^3	0.035
30	35×10^3	0.012

ACETIC ACID AT pH 5.5

0*	120×10^6	100%
5	60×10^5	5.0
10	45×10^4	0.37
15	70×10^3	0.058
20	100×10^2	0.0083
25	220×10^2	0.019
30	55×10^2	0.0046
0*	135×10^6	100%
5	90×10^6	67
10	130×10^4	0.97
15	35×10^3	0.026
20	250×10^2	0.019
25	65×10^2	0.0048
30	100×10^2	0.0074

ACETIC ACID AT pH 5.0

0*	135×10^6	100%
5	280×10^5	21
10	290×10^3	0.22
15	30×10^3	0.022
20	50×10^3	0.036
25	110×10^2	0.0082
30	50×10^2	0.0036
0*	60×10^6	100%
5	70×10^5	12
10	80×10^4	0.13
15	250×10	0.0042
20	30×10^2	0.0050
25	20×10	0.00030
30	9×10	0.00015

ACETIC ACID AT pH 5.0 and

HYDROXYLAMINE (200 ppm)

0*	60×10^6	100%
5	30×10^5	5.0
10	110×10^3	0.18
15	150×10	0.0025
20	120×10^2	0.020
25	85×10	0.0014
30	130×10	0.0022
0*	120×10^6	100%
5	180×10^5	15
10	110×10^4	0.92
15	40×10^3	0.033
20	55×10^2	0.0046
25	240×10	0.0020
30	75×10	0.00062

SODIUM LAURATE (0.075%)

0*	85×10^6	100%
5	65×10^5	7.7
10	35×10^4	0.35
15	260×10^2	0.031
20	125×10^3	0.15
25	210×10	0.0025
30	55×10	0.00065
0*	90×10^6	100%
5	140×10^5	16
10	135×10^4	1.5
15	55×10^3	0.061
20	85×10^2	0.0094
25	40×10^2	0.0044
30	105×10	0.0012

SODIUM LAURATE (0.125%)

0*	90×10^6	100%
5	35×10^5	3.9
10	130×10^4	1.4
15	75×10^3	0.083
20	30×10^2	0.0033
25	295×10	0.0032
30	95×10	0.0011
0*	170×10^6	100%
5	100×10^4	0.59
10	200×10^3	0.12
15	100×10^3	0.059
20	215×10^2	0.013
25	95×10^2	0.0056
30	30×10^2	0.0018
0*	100×10^6	100%
30	250×10	0.0025
0*	85×10^6	100%
5	60×10^5	7.0
10	35×10^4	0.41
15	130×10^3	0.15
20	190×10^2	0.022
25	110×10^2	0.013
30	65×10^2	0.0076

SODIUM LAURATE (0.075%) and
HYDROXYLAMINE (200 ppm)

0*	250×10^6	100%
5	250×10^5	10
10	105×10^4	0.42
15	80×10^4	0.32
20	45×10^3	0.018
25	65×10^2	0.0026
30	225×10	0.00090

0*	85×10^6	100%
5	175×10^5	21
10	120×10^4	1.4
15	170×10^2	0.020
20	75×10^2	0.0088
25	145×10	0.0017
30	60×10	0.00071

SORBIC ACID (0.075%)

0*	115×10^6	100%
5	70×10^6	61
10	180×10^5	16
15	50×10^5	4.4
20	100×10^4	0.87
25	90×10^3	0.078
30	290×10^2	0.025
0*	125×10^6	100%
5	70×10^6	56
10	265×10^5	21
15	45×10^4	0.36
20	125×10^3	0.10
25	40×10^3	0.032
30	135×10^2	0.011
0*	235×10^6	100%
5	95×10^6	40
10	145×10^4	0.62
15	200×10^3	0.085
20	55×10^3	0.023
25	230×10^2	0.0098
30	35×10^2	0.0015

SORBIC ACID (0.125%)

0*	115×10^6	100%
5	45×10^6	39
10	40×10^5	3.5
15	205×10^4	1.8
20	225×10^3	0.20
25	45×10^3	0.039
30	230×10^2	0.020
0*	290×10^6	100%
30	155×10	0.00053
0*	125×10^6	100%
5	35×10^6	28
10	135×10^5	11
15	40×10^4	0.32
20	35×10^3	0.028
25	290×10^2	0.023
30	120×10^2	0.0096

SORBIC ACID (0.075%) and
HYDROXYLAMINE (200 ppm)

0*	140×10^6	100%
5	90×10^5	6.5
10	145×10^4	1.0
15	125×10^3	0.089
20	260×10^2	0.019
25	50×10^2	0.0036
30	95×10	0.00068
0*	235×10^6	100%
5	105×10^6	45
10	60×10^4	0.25
15	90×10^3	0.038
20	130×10	0.00055
25	45×10^2	0.0019

CALCIUM PROPIONATE (0.075%)

0*	200×10^6	100%
5	150×10^6	75
10	130×10^5	6.4
15	50×10^5	2.5
20	90×10^4	0.45
25	135×10^3	0.067
30	75×10^2	0.0038
0*	220×10^6	100%
5	105×10^6	58
10	50×10^5	2.5
15	50×10^4	0.25
20	200×10^3	0.091
25	60×10^3	0.027
30	85×10^3	0.039
0*	250×10^6	100%
10	225×10^5	9.0
15	75×10^4	0.30
20	30×10^4	0.12
25	100×10^3	0.04

CALCIUM PROPIONATE (0.125%)

0*	210×10^6	100%
5	75×10^6	36
10	50×10^5	2.4
15	45×10^4	0.21
20	180×10^3	0.086
25	35×10^3	0.017
30	150×10^2	0.0072

0*	200×10^6	100%
5	140×10^6	70
10	90×10^5	4.5
15	30×10^5	1.5
20	40×10^4	0.20
25	90×10^3	0.045
30	195×10^2	0.0098

CALCIUM PROPIONATE (0.075%) and

HYDROXYLAMINE (200 ppm)

0*	250×10^6	100%
5	235×10^5	9.4
10	140×10^4	0.56
15	210×10^3	0.084
20	50×10^2	0.0020
25	65×10	0.00026
30	10×10	0.000040
0*	220×10^6	100%
5	65×10^6	30
10	85×10^4	0.39
15	275×10^2	0.013
20	50×10^3	0.023
25	50×10^2	0.0023
30	6×10	0.000027
0*	170×10^6	100%
5	45×10^5	2.6
10	250×10^3	0.15
15	210×10^2	0.012
20	165×10^2	0.0097
25	95×10	0.00056
30	50×10	0.00029

CALCIUM PROPIONATE (0.125%) and

HYDROXYLAMINE (200 ppm)

0*	170×10^6	100%
5	125×10^5	7.4
10	235×10^3	0.14
15	40×10^3	0.024
20	55×10^2	0.0032
25	270×10	0.0016
30	30×10	0.00018
0*	210×10^6	100%
5	80×10^5	3.8
10	45×10^4	0.21
15	70×10^3	0.033
20	200×10^2	0.0096
25	35×10	0.00017
30	10×10	0.000048

n-PROPIONIC ACID (0.075%)

0*	35×10^6	100%
5	110×10^5	31
10	160×10^4	4.6
15	250×10^3	0.71
20	35×10^2	0.010
25	100×10^2	0.029
30	85×10	0.0024
0*	90×10^6	100%
5	45×10^6	50
10	240×10^4	2.7
15	160×10^3	0.18
20	95×10^3	0.11
25	35×10^3	0.039
30	70×10^2	0.0078

n-PROPIONIC ACID (0.125%)

0*	90×10^6	100%
5	220×10^5	24
10	260×10^4	3.0
15	30×10^4	0.33
20	135×10^2	0.015
25	250×10	0.0028
30	55×10^2	0.0061
0*	35×10^6	100%
5	130×10^5	37
10	260×10^4	3.0
15	30×10^4	0.33
20	135×10^2	0.015
25	250×10	0.0028
30	55×10^2	0.0061
0*	35×10^6	100%
5	130×10^5	37
10	180×10^4	5.1
15	100×10^3	0.28
20	220×10^2	0.063
25	120×10^2	0.035
30	130×10	0.0037
0*	140×10^6	100%
5	190×10^5	14
10	130×10^4	0.93
15	210×10^3	0.15
20	40×10^3	0.029
25	30×10^2	0.0021
30	110×10	0.00079

n-PROPIONIC ACID (0.075%) and
HYDROXYLAMINE (200 ppm)

0*	90×10^6	100%
5	200×10^5	22
10	100×10^3	0.11
15	110×10^2	0.012
20	100×10	0.0011
25	60×10^2	0.0067
30	3×10	0.000033
0*	90×10^6	100%
5	30×10^6	33
10	145×10^3	0.16
15	30×10^2	0.0033
20	45×10	0.00050
30	50×10	0.00056

n-PROPIONIC ACID (0.125%) and
HYDROXYLAMINE (200 ppm)

0*	90×10^6	100%
5	70×10^5	7.8
10	35×10^3	0.039
15	40×10^2	0.0045
20	70×10	0.00078
25	210×10	0.0023
30	65×10	0.00072
0*	140×10^6	100%
5	180×10^5	13
10	125×10^3	0.089
15	130×10^2	0.0093
20	200×10	0.0014
25	35×10	0.00025
30	10×10	0.000072

L-ASCORBIC ACID (0.10%)

0*	185×10^6	100%
5	40×10^6	22
10	75×10^4	0.41
15	30×10^3	0.016
20	65×10^2	0.0035
25	135×10	0.00073
30	55×10	0.00030
0*	215×10^6	100%
5	280×10^5	13
10	290×10^4	1.3
15	90×10^3	0.042
20	140×10^2	0.0065
25	275×10	0.00013
30	120×10	0.00056
0*	100×10^6	100%
5	245×10^5	25
10	95×10^3	0.095
15	110×10^2	0.011
20	50×10^2	0.0050
25	115×10	0.0012
30	45×10	0.00045
0*	290×10^6	100%
30	210×10	0.00073

L-ASCORBIC ACID (0.10%) and
HYDROXYLAMINE (200 ppm)

0*	85×10^6	100%
5	45×10^5	5.3
10	55×10^4	0.65
15	75×10^3	0.088
20	160×10^2	0.019
25	120×10	0.0014
30	40×10	0.00047

0*	250×10^6	100%
5	35×10^6	14
10	50×10^5	2.0
15	75×10^4	0.30
20	95×10^3	0.038
25	145×10^2	0.0058
30	95×10	0.00038

SODIUM ASCORBATE (0.10%)

0*	130×10^6	100%
5	45×10^6	35
10	70×10^5	5.4
15	200×10^3	0.15
20	65×10^3	0.050
25	180×10^2	0.014
30	65×10^2	0.0050
0*	135×10^6	100%
5	180×10^5	13
10	120×10^4	0.89
15	70×10^2	0.0052
20	215×10^2	0.016
25	35×10^2	0.0026
30	40×10	0.00030
0*	35×10^6	100%
5	250×10^4	7.1
10	60×10^4	1.7
15	80×10^3	0.23
20	115×10^2	0.033
25	50×10^2	0.014
30	45×10	0.0013

SODIUM ASCORBATE (0.10%) and
HYDROXYLAMINE (200 ppm)

0*	90×10^6	100%
5	200×10^4	2.2
10	180×10^3	0.20
15	250×10^2	0.028
25	50×10^2	0.0056
30	30×10^2	0.0033
0*	115×10^6	100%
5	65×10^5	5.7
10	100×10^3	0.087
15	125×10^2	0.011
20	55×10^2	0.0048
25	125×10^2	0.011
30	60×10^2	0.0052
0*	130×10^6	100%
5	45×10^5	3.5
10	55×10^4	0.42
15	70×10^2	0.0054
20	40×10^2	0.0031
25	90×10	0.00069
30	70×10	0.00054

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* Mean of duplicate plate count of two tubes.

3. ANTIBIOTICS

Heating Time (minutes)	Conc. of Antibiotic (ppm)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C.	Percent Survival (%)
		AUREOMYCIN	
ROAST BEEF HOMOGENATE			
0*	0	55×10^6	100%
5	1	45×10^5	8.2
10	1	110×10^2	0.020
15	1	150×10	0.0027
20	1	20×10	0.00036
25	1	4×10	0.000073
30	1	30×10	0.00055
5	10	150×10^5	27%
10	10	80×10^3	0.15
15	10	50×10	0.000091
20	10	0×10	--
25	10	0×10	--
30	10	0×10	--
5	50	170×10^3	0.31%
7.5	50	65×10^2	0.012
10	50	15×10	0.00027
15	50	0×10	--
0*	0	155×10^6	100%
5	1	75×10^5	4.8
10	1	90×10^3	0.058
15	1	230×10^2	0.015
20	1	120×10	0.00078
25	1	135×10	0.00087
30	1	15×10	0.000097

AUREOMYCIN

5	10	135×10^5	8.7%
10	10	50×10^2	0.00032
15	10	0×10	--
20	10	0×10	--
25	10	0×10	--
5	50	85×10^3	0.055%
7.5	50	15×10	0.000097
10	50	0×10	--
0*	0	140×10^6	100%
5	1	250×10^5	18
10	1	90×10^3	0.064
15	1	120×10^2	0.0086
20	1	30×10^2	0.0021
25	1	40×10	0.00029
30	1	10×10	0.000072
5	10	80×10^5	5.7%
10	10	270×10^3	0.19
15	10	40×10^2	0.0029
20	10	1×10	--
5	50	60×10^3	0.043%
7.5	50	35×10^3	0.025
10	50	15×10	0.00011
0*	0	90×10^6	100%
5	1	140×10^5	16
10	1	260×10^2	0.029
15	1	170×10	0.0019
20	1	45×10	0.00050
25	1	20×10	0.00022
30	1	15×10	0.000017

AUREOMYCIN

5	10	170×10^5	19%
10	10	50×10^3	0.055
15	10	45×10	0.00050
20	10	1×10	--
5	50	85×10^4	0.95%
7.5	50	135×10	0.0015
10	50	35×10	0.00039
0*	0	190×10^6	100%
5	10	200×10^5	10
10	10	120×10^3	0.063
15	10	30×10^2	0.0016
20	10	1×10	--
25	10	0×10	--
30	10	1×10	--

* Mean of Duplicate Counts of Duplicate Tubes

Heating Time (minutes)	Conc. of Antibiotic (ppm)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
		AUREOMYCIN	
HAM HOMOGENATE			
0*	0	75×10^6	100%
5	1	110×10^5	15
10	1	50×10^4	0.67
15	1	250×10^2	0.033
20	1	55×10^2	0.0073
25	1	70×10^2	0.0093
30	1	100×10^2	0.013
5	10	150×10^5	20%
10	10	35×10^4	0.47
15	10	40×10^3	0.053
20	10	50×10^2	0.0067
25	10	65×10^2	0.0087
30	10	70×10	0.00094
5	50	170×10^4	2.3%
7.5	50	80×10^3	0.11
10	50	40×10	0.0053
0*	0	160×10^6	100%
5	1	50×10^6	31
10	1	130×10^3	0.081
15	1	180×10^3	0.11
20	1	60×10^3	0.037
25	1	70×10^2	0.0044
30	1	40×10^3	0.025

AUREOMYCIN

5	10	80×10^6	50%
10	10	30×10^4	0.19
15	10	65×10^3	0.041
20	10	260×10^2	0.016
25	10	40×10^2	0.0025
30	10	45×10^2	0.0028
5	50	70×10^5	4.4%
7.5	50	-	-
10	50	65×10^3	0.041
0*	0	130×10^6	100%
5	1	55×10^5	42
10	1	220×10^3	0.21
15	1	50×10^3	0.038
20	1	80×10^3	0.062
25	1	35×10^3	0.027
30	1	45×10^2	0.0035
5	10	40×10^5	31%
10	10	50×10^4	0.38
15	10	220×10^2	0.017
20	10	110×10^2	0.0085
25	10	125×10	0.00096
30	10	90×10^2	0.0069
5	50	95×10^5	7.3%
7.5	50	90×10^3	0.069
10	50	40×10^3	0.031
15	50	10×10	0.000085

AUREOMYCIN

0*	0	150×10^6	100%
5	1	190×10^5	13
10	1	60×10^4	0.40
15	1	40×10^3	0.027
20	1	130×10^3	0.087
25	1	190×10^2	0.013
30	1	140×10	0.00093
5	10	140×10^5	9.3%
10	10	120×10^3	0.080
15	10	135×10^3	0.090
20	10	60×10^2	0.0040
25	10	260×10^2	0.017
30	10	35×10	0.00023
5	50	200×10^4	1.3%
7.5	50	65×10^4	0.43
10	50	160×10^2	0.011
15	50	45×10	0.00030

* Mean of Duplicate Counts of Duplicate Tubes.

Heating Time (minutes)	Conc. of Antibiotic (ppm)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
		TERRAMYCIN	
ROAST BEEF HOMOGENATE			
0*	0	50×10^6	100%
5	1	120×10^5	24
10	1	190×10^2	0.038
15	1	80×10	0.0016
20	1	100×10	0.0020
25	1	50×10^2	0.010
30	1	150×10	0.0030
5	10	40×10^5	8.0%
10	10	60×10^2	0.012
15	10	150×10	0.0030
20	10	105×10	0.0021
25	10	75×10	0.0015
30	10	25×10	0.00050
5	50	45×10^4	0.90
10	50	50×10^2	0.01
15	50	125×10	0.0025
20	50	1×10	--
0*	0	80×10^6	100%
5	1	55×10^5	6.9
10	1	110×10^3	0.14
15	1	40×10^2	0.0050
20	1	230×10	0.0029
25	1	30×10	0.00038
30	1	150×10	0.0019

TERRAMYCIN

5	10	85×10^5	11%
10	10	250×10^3	0.31
15	10	75×10^2	0.0084
20	10	280×10	0.0035
25	10	120×10	0.0015
30	10	70×10^2	0.0088
5	50	100×10^4	1.3%
10	50	130×10^2	0.016
15	50	45×10	0.00051
20	50	8×10	0.00010
0*	0	90×10^6	100%
5	1	95×10^6	11
10	1	55×10^3	0.061
15	1	185×10^2	0.021
20	1	50×10^2	0.0051
25	1	140×10	0.0016
30	1	30×10	0.00033
5	10	180×10^5	20%
10	10	65×10^3	0.072
15	10	130×10^2	0.014
20	10	60×10^2	0.0067
25	10	85×10	0.00094
30	10	100×10	0.0011
5	50	35×10^5	3.9%
10	50	190×10	0.0021
15	50	90×10	0.0010
20	50	0×10	--

TERRAMYCIN

0*	0	120×10^6	100%
5	1	60×10^6	50
10	1	105×10^2	0.0088
15	1	40×10^2	0.0033
20	1	40×10^3	0.033
25	1	60×10	0.00050
30	1	190×10	0.0016
5	10	45×10^6	38%
10	10	270×10^2	0.023
15	10	40×10^3	0.033
20	10	65×10^2	0.0054
25	10	250×10	0.0021
30	10	40×10	0.00033
5	50	70×10^5	5.8%
10	50	180×10^2	0.015
15	50	30×10	0.00025
20	50	10×10	0.000083

HAM HOMOGENATE

0*	0	100×10^6	100%
5	1	250×10^5	25
10	1	60×10^4	0.60
15	1	190×10^2	0.019
20	1	95×10^2	0.0095
25	1	150×10	0.0015
30	1	260×10	0.0024
5	10	70×10^5	7.0%
10	10	190×10^3	0.19
15	10	240×10^2	0.024
20	10	40×10^2	0.0040
25	10	150×10	0.0015
30	10	65×10	0.00065

TERRAMYCIN

5	50	30×10^5	3.0%
10	50	160×10^3	0.16
15	50	280×10^2	0.028
20	50	70×10	0.00070
0*	0	85×10^6	100%
5	1	90×10^5	11
10	1	95×10^4	1.1
15	1	70×10^3	0.082
20	1	30×10^2	0.0035
25	1	120×10^2	0.014
30	1	60×10	0.00071
5	10	85×10^5	10%
10	10	75×10^4	0.88
15	10	75×10^3	0.088
20	10	180×10^2	0.021
25	10	50×10^2	0.0059
30	10	100×10	0.0012
5	50	190×10^4	2.2%
10	50	220×10^3	0.26
15	50	190×10^2	0.022
20	50	90×10	0.0011
0*	0	130×10^6	100%
5	1	85×10^5	6.5
10	1	40×10^5	3.1
15	1	35×10^4	0.27
20	1	95×10^2	0.0073
25	1	75×10^2	0.0058
30	1	190×10	0.0015

TERRAMYCIN

5	10	30×10^6	23%
10	10	55×10^4	0.42
15	10	70×10^3	0.054
20	10	85×10^2	0.0065
25	10	35×10^2	0.0027
30	10	110×10	0.00085
5	50	90×10^5	6.9%
10	50	170×10^3	0.13
15	50	210×10^2	0.016
20	50	30×10	0.00023
0*	0	165×10^6	100%
5	1	35×10^6	21
10	1	200×10^4	1.2
15	1	170×10^3	0.10
20	1	40×10^3	0.024
25	1	290×10^2	0.018
30	1	35×10^2	0.0021
5	10	260×10^5	16%
10	10	130×10^4	0.79
15	10	290×10^2	0.018
20	10	50×10^2	0.0030
25	10	60×10^2	0.0036
30	10	35×10^2	0.0021
5	50	80×10^5	4.8%
10	50	50×10^4	0.30
15	50	70×10^2	0.0042
20	50	50×10	0.00030

* Mean of Duplicate Counts of Duplicate Tubes

Heating Time (minutes	Conc. of Antibiotic (ppm)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
		SUBTILIN	
ROAST BEEF HOMOGENATE			
0*	0	130×10^6	100%
5	1	140×10^5	11
10	1	190×10^2	0.015
15	1	280×10^2	0.022
20	1	60×10^2	0.0046
25	1	35×10^3	0.027
30	1	70×10^2	0.0054
5	10	40×10^4	0.31%
10	10	50×10	0.00039
15	10	110×10	0.00085
20	10	30×10	0.00023
25	10	2×10	0.000015
30	10	0×10	--
5	50	35×10^3	0.027%
7.5	50	25×10	0.00019
10	50	20×10	0.00015
15	50	0×10	--
0*	0	55×10^6	100%
5	1	150×10^5	27
10	1	75×10^2	0.013
15	1	280×10	0.0051
20	1	95×10	0.0017
25	1	10×10	0.00018
30	1	20×10	0.00036

SUBTILIN

5	10	30×10^4	0.55%
10	10	30×10	0.00055
15	10	15×10	0.00027
20	10	5×10	0.000091
25	10	0×10	--
30	10	0×10	--
5	50	35×10^2	0.0064%
7.5	50	40×10	0.00073
10	50	5×10	0.000097
0*	0	90×10^6	100%
5	1	180×10^5	20
10	1	35×10^3	0.039
15	1	30×10^2	0.0033
20	1	60×10^2	0.0067
25	1	135×10	0.0015
30	1	170×10	0.0019
5	10	80×10^4	0.89%
10	10	20×10	0.00022
15	10	15×10	0.00017
20	10	0×10	--
25	10	0×10	--
30	10	0×10	--
5	50	175×10	0.0019%
7.5	50	15×10	0.00017
10	50	2×10	0.000022

		SUBTILIN	
0*	0	45×10^6	100%
5	1	40×10^5	8.9
10	1	230×10^2	0.051
15	1	75×10	0.0017
20	1	65×10^2	0.014
25	1	190×10	0.0042
30	1	40×10	0.00089
5	10	35×10^3	0.078%
10	10	95×10	0.0021
15	10	65×10	0.0014
20	10	60×10	0.0013
25	10	5×10	0.00011
30	10	0×10	--
5	50	40×10^3	0.089%
7.5	50	25×10	0.00060
10	50	5×10	0.000022
15	50	0×10	--

HAM HOMOGENATE

0*	0	230×10^6	100%
5	1	70×10^6	31
10	1	55×10^4	0.24
15	1	135×10^3	0.059
20	1	40×10^3	0.017
25	1	65×10^3	0.028
30	1	240×10^2	0.010

SUBTILIN			
5	10	165×10^5	7.2%
10	10	110×10^3	0.048
15	10	210×10^2	0.0091
20	10	180×10	0.00078
25	10	40×10^2	0.0017
30	10	60×10	0.00026
5	50	160×10^3	0.070%
7.5	50	200×10	0.00087
10	50	35×10	0.00015
15	50	0×10	--
0*	0	65×10^6	100%
5	1	40×10^5	6.2%
10	1	220×10^2	0.034
15	1	50×10^3	0.077
20	1	60×10^3	0.092
25	1	40×10^3	0.062
30	1	165×10^2	0.022
5	10	35×10^5	5.4%
10	10	95×10^2	0.015
15	10	105×10^2	0.016
20	10	30×10	0.00046
25	10	15×10	0.00023
30	10	5×10	0.000079
5	50	235×10^3	0.036%
7.5	50	10×10	0.00015
10	50	0×10	--
15	50	0×10	--

SUBTILIN

0*	0	110×10^6	100%
5	1	30×10^6	27
10	1	130×10^3	0.12
15	1	280×10^2	0.025
20	1	45×10^3	0.041
25	1	170×10^2	0.015
30	1	120×10^2	0.011
5	10	270×10^4	2.5%
10	10	230×10^2	0.021
15	10	65×10^2	0.0059
20	10	40×10^2	0.0037
25	10	20×10	0.00018
30	10	35×10	0.00032
5	50	260×10	0.0024%
7.5	50	240×10	0.0022
10	50	0×10	--
0*	0	120×10^6	100%
5	1	180×10^5	15
10	1	90×10^3	0.075
15	1	60×10^3	0.050
20	1	290×10^2	0.024
25	1	40×10^3	0.033
30	1	65×10^3	0.054
5	10	195×10^4	1.6%
10	10	45×10^2	0.0037
15	10	30×10^2	0.0025
20	10	170×10	0.0014
25	10	35×10	0.00029
30	10	20×10	0.00017

SUBTILIN			
5	50	65×10^2	0.0054%
7.5	50	75 x 10	0.00063
10	50	6 x 10	0.000050
15	50	0 x 10	--

* Mean of duplicate counts of duplicate tubes.

Heating Time (minutes)	Conc. of Antibiotic (ppm)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
		NISIN	
ROAST BEEF HOMOGENATE			
0*	0	110×10^6	100%
5	1	35×10^5	3.2
10	1	75×10^3	0.068
15	1	35×10^2	0.0032
20	1	70×10^2	0.0064
25	1	90×10^2	0.0082
30	1	30×10^2	0.0027
5	10	150×10^4	1.4%
10	10	85×10^2	0.0077
15	10	260×10	0.0024
20	10	110×10	0.0010
25	10	60×10	0.00055
30	10	20×10	0.00018
5	50	70×10^2	0.0064%
7.5	50	40×10^2	0.0036
10	50	20×10	0.00018
0*	0	100×10^6	100%
5	1	40×10^6	42
10	1	30×10^3	0.032
15	1	250×10^2	0.026
20	1	160×10^2	0.017
25	1	35×10^2	0.0037
30	1	40×10^2	0.0042

NISIN			
5	10	60×10^5	6.3%
10	10	250×10	0.0026
15	10	70×10^2	0.0074
20	10	110×10	0.0012
25	10	150×10	0.0016
30	10	30×10	0.00032
5	50	110×10^2	0.012%
7.5	50	75×10	0.00079
10	50	15×10	0.00016
0*	0	130×10^6	100%
5	10	120×10^5	9.2%
10	10	210×10^2	0.016
15	10	110×10^2	0.0085
20	10	30×10^2	0.0023
25	10	35×10	0.00027
30	10	15×10	0.00012
0*	0	110×10^6	100%
5	10	220×10^4	2.0
10	10	35×10^2	0.0032
15	10	105×10	0.00096
20	10	50×10	0.00045
25	10	15×10	0.00014
30	10	45×10	0.00041

NISIN			
HAM HOMOGENATE			
0*	0	105×10^6	100%
5	1	35×10^6	33
10	1	150×10^3	0.14
15	1	230×10^2	0.022
20	1	110×10^2	0.010
25	1	50×10^2	0.0048
30	1	40×10^2	0.0038
5	10	55×10^5	5.2%
10	10	200×10^2	0.018
15	10	90×10^2	0.0082
20	10	30×10^2	0.0027
25	10	180×10	0.0017
30	10	150×10	0.0014
5	50	150×10^3	0.14%
7.5	50	175×10^2	0.017
10	50	20×10	0.00019
0*	0	110×10^6	100%
5	1	60×10^5	5.5
10	1	130×10^3	0.12
15	1	70×10^3	0.064
20	1	280×10^2	0.025
25	1	190×10	0.0017
30	1	30×10^2	0.0027
5	10	90×10^5	8.2%
10	10	50×10^3	0.045
15	10	110×10	0.0010

NISIN			
20	10	100 x 10	0.00091
25	10	15 x 10	0.00014
30	10	40 x 10	0.00036
5	50	35 x 10^2	0.032%
7.5	50	120 x 10	0.0011
10	50	55 x 10	0.00050
0*	0	55 x 10^6	100%
5	10	95 x 10^5	17
10	10	50 x 10^3	0.090
15	10	260 x 10	0.0047
20	10	55 x 10^2	0.010
25	10	30 x 10	0.00054
30	10	10 x 10	0.00018
0*	0	100 x 10^6	100%
5	10	50 x 10^5	5.0
10	10	30 x 10^3	0.030
15	10	35 x 10^2	0.0035
20	10	150 x 10	0.0015
25	10	230 x 10	0.0023
30	10	80 x 10	0.00080

* Mean of duplicate counts of duplicate tubes

E.

TABLES OF SURVIVOR COUNTS OF AN ENTEROTOXIN PRODUCING STRAIN OF MICROCOCCUS PYOGENES, VAR. AUREUS ON UNSTERILIZED SLICES OF ROAST BEEF AND BOILED HAM IRRADIATED WITH 1 M.e.v. CATHODE RAYS

a. ROAST BEEF SLICES

Irradiation Dose x 10^3 rep (93)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
0	255×10^5	100%
100	60×10^5	24
200	50×10^4	2.0
400	40×10^4	1.6
600	270×10^2	0.11
800	70×10^2	0.027
1000	80×10^2	0.031
1500	75×10	0.0029
0	195×10^6	100%
100	245×10^5	13
200	95×10^4	4.9
800	125×10^4	0.64
1000	55×10^3	0.028
1500	100×10	0.00057
0	160×10^6	100%
100	115×10^5	7.2
100	230×10^4	1.4
200	220×10^4	1.4
200	105×10^4	0.66
400	75×10^4	0.47
400	100×10^4	0.63
400	65×10^4	0.41
600	50×10^4	0.31
600	115×10^3	0.072
600	75×10^3	0.047
800	90×10^2	0.0056

b. BOILED HAM SLICES

Irradiation Dose $\times 10^3$ rep (93)	Mean of Duplicate T.G.E. Plate Counts Per Gram at 37°C	Percent Survival (%)
0	70×10^6	100%
200	35×10^4	0.50
400	270×10^3	0.39
400	60×10^3	0.006
600	250×10^2	0.036
600	40×10^3	0.056
800	220×10^3	0.31
800	280×10^2	0.040
1000	60×10^3	0.086
1000	180×10^2	0.026
1500	55×10	0.00079
1500	120×10	0.0017
0	170×10^5	100%
100	265×10^4	16
100	30×10^5	18
200	75×10^4	4.4
200	70×10^4	4.1
400	50×10^4	2.9
400	90×10^4	5.3
600	230×10^3	1.4
600	35×10^3	0.21
800	180×10^2	0.11
0	85×10^6	100%
100	110×10^5	13
200	135×10^4	1.6
800	110×10^2	0.013
1000	35×10^3	0.041
1500	115×10	0.014

F.

TABLES OF SURVIVOR COUNTS OF AN ENTEROTOXIN PRODUCING STRAIN OF MICROCOCCUS PYOGENES, VAR. AUREUS ON UNSTERILIZED SLICES OF ROAST BEEF AND BOILED HAM FROZEN AND HELD FOR 24 HOURS AT 0°F AFTER HEAT TREATMENT AT 185°F OR IRRADIATION WITH 1 M.e.v. CATHODE RAYS

1. FREEZING OF SLICES PREVIOUSLY HEATED AT 185°F

Heating Time (minutes)	Mean of Duplicate T.G.E. Plate Counts: Before Freezing	Mean of Duplicate T.G.E. Plate Counts: After Freezing	Reduction in Population	Percent Reduction (%)	
a. ROAST BEEF SLICES					
0	65×10^6	210×10^5	44×10^6	68	
0	250×10^5	60×10^5	190×10^5	76	
0	250×10^5	100×10^5	150×10^5	60	
			MEAN		68%
5	170×10^5	70×10^5	100×10^5	59	
5	75×10^4	250×10^3	50×10^4	71	
5	75×10^4	280×10^3	47×10^4	67	
			MEAN		66%
10	35×10^4	100×10^3	25×10^4	71	
10	110×10^2	250×10	85×10^2	77	
10	110×10^2	80×10^2	30×10^2	27	
			MEAN		58%
15	115×10^3	75×10^3	40×10^3	35	
15	95×10^2	45×10^2	50×10^2	53	
15	95×10^2	35×10^2	60×10^2	68	
			MEAN		51%

Heating Time (Minutes)	Mean of Duplicate T.G.E. Plate Counts		Reduction in Population	Percent Reduction (%)
	Before Freezing	After Freezing		
b. BOILED HAM SLICES				
0	135×10^6	65×10^6	70×10^6	52
0	140×10^5	40×10^5	100×10^5	71
0	240×10^5	130×10^5	110×10^5	44
			MEAN	56%
5	65×10^6	30×10^6	35×10^6	54
5	100×10^5	55×10^5	45×10^5	45
5	70×10^5	40×10^5	30×10^5	42
			MEAN	47%
10	60×10^4	250×10^3	35×10^4	58
10	30×10^2	90×10	21×10^2	70
10	45×10^2	270×10	18×10^2	40
			MEAN	56%
15	60×10^2	100×10	50×10^2	83
15	12×10	40×10^2	---	--
15	65×10	20×10	45×10	69
			MEAN	76%

2. FREEZING OF SLICES PREVIOUSLY IRRADIATED AT 1 m.e.v.

Dose ($\times 10^3$ rep_{93})	Mean of Duplicate T.G.E. Plate Counts: Before Freezing	Mean of Duplicate T.G.E. Plate Counts: After Freezing	Reduction in Population	Percent Reduction (%)
a. ROAST BEEF SLICES				
0	255×10^5	220×10^5	35×10^5	14
0	255×10^5	115×10^5	140×10^5	55
0	195×10^6	155×10^6	40×10^6	21
			MEAN	30%
200	50×10^4	210×10^3	29×10^4	58
200	50×10^4	190×10^3	31×10^4	62
200	95×10^4	250×10^3	70×10^4	74
			MEAN	65%
400	40×10^4	190×10^3	21×10^4	52
400	40×10^4	30×10^4	10×10^4	24
400	75×10^4	45×10^4	30×10^4	40
			MEAN	38%
800	70×10^2	50×10	65×10^2	93
800	70×10^2	150×10	55×10^2	79
800	90×10^2	130×10	77×10^2	86
			MEAN	86%
1500	75×10	25×10	50×10	67
1500	75×10	65×10	10×10	40
1500	100×10	40×10	60×10	60
			MEAN	55%

Dose ($\times 10^3$ rep_{93})	Mean of Duplicate T.G.E. Plate Counts: Before Freezing	Mean of Duplicate T.G.E. Plate Counts: After Freezing	Reduction in Population	Percent Reduction (%)
b. BOILED HAM SLICES				
0	70×10^6	40×10^6	30×10^6	43
0	70×10^6	280×10^5	42×10^6	60
0	85×10^6	100×10^5	75×10^6	88
			MEAN	64%
200	70×10^4	40×10^4	30×10^4	43
200	75×10^4	190×10^3	56×10^4	75
200	135×10^4	60×10^4	75×10^4	56
			MEAN	58%
400	50×10^4	180×10^3	32×10^4	64
400	270×10^3	95×10^3	175×10^3	65
400	60×10^3	80×10^2	52×10^3	87
			MEAN	72%
800	220×10^3	105×10^3	115×10^3	52
800	280×10^2	60×10^2	220×10^2	79
800	110×10^2	45×10^2	65×10^2	59
			MEAN	63%
1500	55×10	40×10	15×10	26
1500	115×10^2	75×10^2	40×10^2	35
1500	120×10	45×10	75×10	63
			MEAN	42%

G.

SAMPLE CALCULATIONS

PROCEDURES FOR REGRESSION ANALYSES FROM

Patterson, D.D.
Statistical Technique in Agricultural Research
McGraw-Hill Book Co., New York, 1939

1. Regression Coefficient

The equation for linear regression is

$$X = M_x + b_{xy}(y - M_y),$$

where, X is the average value of the x variates that may be expected when the value of the y variable is fixed at y. M_x and M_y are the means of the x and y variables, respectively. b_{xy} is the regression coefficient of x on y, i.e., the mean number of units the x variable will change for a unit change in the y variable.

The data is arranged in the following table for calculation of b_{xy}. This data was found in Section IV-A for the survival rate of Micrococcus pyogenes, var. aureus, Strain 209, suspended in 2.0 ml of ham homogenate with an aliquot of 0.5 ml of sterile, distilled water added, and heated at 140°F. The curve drawn from this data was the control curve for evaluating survival curves influenced by the addition of 0.5 ml of chemical or anitbiotic substances to 2.0 ml of the inoculated suspension. The data is listed in Section C, Appendix.

Heating Time (min)		Mean Survival (%)			
y	y^2	S	x = log S	x^2	xy
5	25	38	1.580	2.49	7.89
10	100	5.2	0.716	0.52	7.16
15	225	0.68	-0.168	0.03	-2.52
20	400	0.15	-0.826	0.68	-16.52
25	625	0.041	-1.389	1.92	-34.73
30	900	0.013	-1.892	3.57	-56.76
105	2275	44.083	-1.979	9.21	-95.48

$$\text{Sum of Squares of X(S.S.x)} = x^2 - \frac{(x)^2}{N}$$

$$= 9.21 - \frac{(-1.979)^2}{6}$$

$$= 8.56$$

$$S.S.y = y^2 - \frac{(y)^2}{N}$$

$$= 2275 - \frac{(105)^2}{6}$$

$$= 442$$

$$\text{Sum of Products, } xy = xy - \frac{(x)(y)}{N}$$

$$= -95.48 - \frac{(-1.979)(105)}{6}$$

$$= -60.69$$

$$\text{Regression Coefficient, } b_{xy} = \frac{-60.69}{442} = -0.138$$

The following table shows the treatment of data from section IV(d), listed in Section C, Appendix. This survival data was from the substitution of an enterotoxin producing strain of Micrococcus pyogenes, var. aureus for the F.D.A. 209 strain described in the previous procedure.

y	y^2	S	x = log S	x^2	xy
5	25	18	1.256	1.57	6.27
10	100	2.1	0.322	0.11	3.22
15	225	0.39	-0.408	0.17	-6.12
20	400	0.14	-0.853	0.73	-17.08
25	625	0.086	-1.066	1.12	-26.65
30	900	0.010	-1.900	3.60	-57.00
105	2275		-2.649	7.30	-97.38

$$SS_x = 7.30 - \frac{(-2.649)^2}{6} \qquad SS_y = 2275 - \frac{(-105)^2}{6}$$

$$= 6.15 \qquad = 442$$

$$\text{Sum of Products, } xy = -97.38 - \frac{(2.649)(105)}{6}$$

$$= -51.09$$

$$b_{xy} = \frac{-51.09}{442} = -0.115$$

2. Points of Regression Curve

Points of the regression curve are found by substitution in the equation

$$X = M_x + b_{xy}(y - M_y)$$

This is shown for both sets of data.

(a) For Strain F.D.A. 209,

$$X = \frac{-1.979}{6} + (-0.137)(y - \frac{105}{6})$$

The points on the regression curve are:

Heating Time (min)	(X)	Survival (%)
5	1.387	24.5
10	0.702	5.04
15	0.022	1.05
20	0.768	0.171
25	-1.348	0.0449
30	-2.038	0.00917

(b) For the enterotoxin producing strain,

$$X = \frac{-2.649}{6} + (-0.115)(y - \frac{105}{6})$$

5	1.00	10.0
10	0.43	2.69
15	-0.14	0.725
20	-0.72	0.191
25	-1.29	0.0513
30	-1.87	0.0135

3. Test for Significance of Regression Coefficient

Each regression curve may be statistically treated to evaluate the significance of the regression coefficient. The variance of b_{xy} is determined from the degrees of freedom of the regression coefficient, the sum of the squares of the independent variable y, and the sum of squares of the deviations of the plotted points from the line of regression. The latter is computed by subtracting the sum of squares of the deviations of each recorded value of x from its mean value X. The sum of squares requires is

$$(x - X)^2$$

This may be computed from the expression

$$(x - X)^2 = S.S.x - (b_{xy}^2)(S.S.y)$$

The standard error (S.E.) of b_{xy} = $\dfrac{(x - X)^2}{(N-2)(S.S.y)}$

The significance of b_{xy} is then determined by calculating t.

$$t = \frac{b_{xy}}{\text{standard error of } b_{xy}}$$

From the table of t values, for N degrees of freedom, the corresponding probability is found.

(a) For Strain F.D.A. 209,

$$(x - X)^2 = 8.56 - (-0.138)^2(442)$$
$$= 0.16$$

$$S.E.\ b_{xy} = m \sqrt{\frac{0.16}{(4)(442)}}$$
$$= 0.0095$$

$$t = \frac{b_{xy}}{S.E.b_{xy}} = \frac{0.138}{0.0095} = 14.5$$

The probability of exceeding this value of t purely by chance is less than 0.01, showing the regression coefficient to be significant.

(b) For the enterotoxin producing strain,

$$(x - X)^2 = 6.15 - (-0.115)^2(442)$$
$$= 0.33$$

$$S.E.\ b_{xy} = \sqrt{\frac{0.33}{(4)(442)}}$$
$$= 0.0137$$

$$t = \frac{0.115}{0.0137} = 8.40$$

The corresponding probability is less than 0.01, proving the significance of the regression coefficient.

4. Test for Significant Difference Between Two Regression Curves

The test used to compare two regression curves is quite similar to that used for testing the significance of the single regression function.

For the two sets of data used in these sample calculations,

Overall $(x - X)^2 = 0.16 + 0.33$ with $4 + 4$ degrees of freedom

$= 0.49$

b_{xy} for strain F.D.A. 209 $= -0.138$

b_{xy} for enterotoxin producing strain $= -0.115$

Difference, D $= 0.023$

The standard error of each value of $b_{xy} = \frac{0.49}{(4)(442)}$

$= 0.0170$

The standard error of D $= (2)(0.0170)$

$= 0.0340$

$$t \text{ value} = \frac{D}{E_D} = \frac{0.023}{0.034} = 0.677$$

The probability of difference at 0.01 is $t = 2.306$. Thus there is no difference between these curves, indicating the similarity in lethality between the two strains at 140°F.

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B I O G R A P H I C A L S K E T C H

BIOGRAPHICAL SKETCH

Lawrence S. Spiegel was born in Salem, Massachusetts on March 18, 1930. He was educated in the Salem school system, and was graduated from the Salem Classical and High School in 1947. He entered The Citadel where he majored in biology-chemistry, and earned the degree of Bachelor of Science in 1951. In the fall of 1951, he began graduate studies in the Division of Medical Sciences at Boston University, and concurrently worked for the Gastroenterology Department, Massachusetts Memorial Hospitals on medical problems arising from X-ray irradiation. He received the Master of Arts degree in Biochemistry in 1953.

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