AN ABSTRACT OF THE THESIS OF

ROBERT ROY WILLIAMS for the MASTER OF SCIENCE
(Name) (Degree)

in MICROBIOLOGY presented on August 10, 1972
(Major) (Date)

Title: THE EFFECT OF ADDED SALT ON THE HEAT RESISTANCE OF SALMONELLA AND ARIZONA SPECIES IN EGGS AND OTHER MEDIA

Abstract approved: Redacted for Privacy
Dr. Paul R. Elliker

The heat resistances of several Salmonella and Arizona laboratory isolated and reference strains have been comparitively studied in Trypticase Soy Broth - Yeast Extract (TSB-YE, 57° C) and liquid whole egg (57° C, 63.5° C) with and without added NaCl. It was determined that Salmonella and Arizona strains had similar heat resistance characteristics in unsalted media but the Arizona neotype strain had significantly greater heat resistance in media with 10.0% NaCl than the other test strains. Construction of survival curves for the Salmonella and Arizona strains in TSB-YE and liquid whole egg revealed that 10.0% NaCl induced nonlogarithmic multiorder thermal death kinetics. The greater heat resistance of the Arizona neotype strain was related to the failure of 10.0% NaCl to induce a "heat sensitive" population as it did in the less heat resistant Salmonella and Arizona strains.

The extended survival of a small number of heat resistant cells of three test strains, particularly the Arizona neotype strain, revealed the inadequacy of the present pasteurization time and temperature requirements for liquid whole egg with 10.0% NaCl. The common methods of calculating D values and extrapolating survival curves used in heat resistance studies and thermal processing of foods are challenged. It is suggested that the failure to determine the small heat resistant population of salmonellae or arizonae that might survive thermal processing could lead to inaccurate estimation of thermal processing requirements, particularly for foods containing NaCl or low a_w, with serious public health consequences.

The Effect of Added Salt on the Heat Resistance of *Salmonella* and *Arizona* Species in Eggs and Other Media

by

Robert Roy Williams

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1973

APPROVED:

Redacted for Privacy

Professor of Microbiology
in charge of major

Redacted for Privacy

Chairman of Department of Microbiology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented August 10, 1972

Typed by Susie Kozlik for Robert Roy Williams

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to Dr. Paul R. Elliker and Mr. Virgil M. Hiatt for the guidance and support that made this thesis possible; to Mr. Michael H. Wehr, Mr. Juan F. Muniz and Mr. John R. Stroup for their excellent and generous technical assistance; to Dr. Hans Lineweaver, Dr. John A. Garibaldi and Mr. Kosuke Ijichi for technical instruction and bacterial cultures; and special thanks to Mr. Roy W. Scheufele for his example to follow.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

THE EFFECT OF ADDED SALT ON THE HEAT RESISTANCE OF SALMONELLA AND ARIZONA SPECIES IN EGGS AND OTHER MEDIA

INTRODUCTION

In 1971 several Salmonella and Arizona strains were isolated by the Oregon State Department of Agriculture (OSDA) Laboratory from liquid whole eggs containing 10.0% NaCl. These pathogenic bacteria were isolated from eggs that had been pasteurized, and re-pasteurized at the required temperature and time of 146° F (63.5° C) for 3.5 minutes, as well as eggs that had not received heat treatment.

The Arizona group is closely related biochemically and serologically to the Salmonella group and is considered pathogens that cause disease in humans that is clinically indistinguishable from salmonellosis. There is considerable disagreement concerning their taxonomic relationship with the Salmonella group. A thorough search of the literature reveals the absence of Arizona heat resistance data. Review of the pasteurization requirement for liquid whole egg containing 10.0% NaCl indicates that the temperature of pasteurization was extrapolated from liquid egg yolk containing 10.0% NaCl data. There is disagreement concerning the temperature and time required to pasteurize both of these egg products.

It is the purpose of this study to characterize NaCl induction of Salmonella and Arizona heat resistance in liquid whole eggs and other media.

LITERATURE REVIEW

Occurrence and Distribution of Salmonella and the Arizona Group

Most of the Salmonella serotypes that have become relatively more prominent in recent years have been associated with poultry, chicken eggs, or egg products, according to the Committee on Salmonella, National Research Council (40). The frequency with which salmonellae are isolated from domestic fowl indicates that these animals constitute the largest single reservoir of these pathogenic bacteria. Commercially processed foods other than poultry and egg products are rarely incriminated (20).

Arizona serotypes are found in a large segment of man's environment (10, 35). Martin, Fife, and Ewing (35), in a 1967 study of Arizona distribution, reported that turkeys comprised approximately fifty percent of the total foci and total numbers of Arizona cultures received by the Center for Disease Control (CDC) during the period of July 1, 1948 through December 31, 1966. Reptiles, man, and chickens were sources of the remainder of the strains submitted. Five percent of the total foci were food products. According to Edwards, Fife, and Ramsey (10), the seeming disparity of incidence of the Arizona in turkeys and chickens is probably more apparent than real. Many Arizona serotypes have been isolated from egg powder that have not been isolated from fowls, indicating that chickens have not been sufficiently examined to determine the true incidence of the arizonae. The number of Arizona cultures recovered from man has increased over the years to approximately twenty percent of the Arizona cultures submitted to CDC during the period of June, 1959, to December, 1966. Dauer (9) estimated that only one percent of human infections are reported to public health authorities.

Definition of the Arizona Group

There has been considerable disagreement concerning the taxonomic relationship within the Family Enterobacteriaceae and subsequent nomenclature of the Salmonella and Arizona groups 5, 11, 13, 16, 29, 30). However, the fact that the Arizona are biochemically and serologically related to the Salmonella and produce disease that is clinically indistinguishable from salmonellosis has remained unquestioned (11, 17, 18, 19, 27, 31, 34, 35). The taxonomic question of whether the Arizona group constitutes the genus Arizona or the subgenus Salmonella arizonae of the genus Salmonella has not been resolved by the appropriate committee of the International Association of Microbiological Societies (IAMS, 28).

The opposing classification schemes are those proposed by Ewing (13) and Kauffmann (29) which have been revised by their respective authors (15, 16, 31). According to Kauffmann (31), the basis of the taxonomic conflict appears to be a clash between two approaches to bacterial classification which can be characterized as the orthodox and the modern classifications. In the orthodox classification, as represented by Ewing, the species and genera are defined by biochemical methods only: the Arizona group is biochemically defined as the genus Arizona within the tribe Salmonelleae. In the modern classification, as represented by Kauffmann, the genera are differentiated biochemically and species are defined biochemically and serologically as a group of related serofermentative phage-types (sero = serological, fermentative = biochemical capabilities, phage-type = infection of the bacterial cell by a specific bacterial virus): the Arizona group is defined as subgenus III, Salmonella arizonae, of the genus Salmonella. In contrast to the genera and subgenera which are defined biochemically by both Ewing and Kauffmann, the Kauffmann classification emphasizes serological identification in the definition of species in that each Salmonella serotype is a species. Further complication is engendered by the use of separate Salmonella and Arizona serological identification schemes (18, 29). Relevant portions of the two opposing classification schemes are presented in Table 1.

The taxonomic proposals of Ewing and Kauffmann have been submitted to the IAMS Enterobacteriaceae subcommittee of the International Committee on Bacteriological Nomenclature (28). Recent

Table 1. Opposing Classification Schemes for Salmonella and Arizona Groups.

A. Ewing Scheme, 1967 (16)

- Family: Enterobacteriaceae
  - Tribe III: Salmonelleae
    - Genus I: Salmonella
      - Species:
        1. Salmonella cholerae - suis
        2. Salmonella typhi
        3. Salmonella enteritidis: all serotypes other than cholerae - suis and typhi
    - Genus II: Arizona
      - Species:
        1. Arizona hinshawii, 1969 (14)
    - Genus III: Citrobacter
      - Species:
        1. Citrobacter freundii

B. Kauffmann Scheme, 1966 (31)

- Family: Enterobacteriaceae
  - Tribe A: Eschericheae
    - Genus I: Escherichia
    - Genus II: Shigella
    - Genus III: Salmonella
      - subgenera: I, II, III, IV
        - subgenus III: Salmonella arizonae - the Arizona group
    - Genus IV: Citrobacter

information indicates that a final decision concerning Salmonella and Arizona has not been made (4, 5 , 28). At present, CDC and the American Society for Microbiology (ASM) endorse the Ewing scheme while the United States Department of Agriculture (USDA) recommends the Kauffmann scheme (2, 26, 47).

Pathogenicity of the Arizona Group

The Arizona group contains recognized pathogens of man and other animals which constitute a public health hazard (10, 11, 19, 27, 31, 35, 39). Edwards and Ewing (11) have stated that since Arizona strains are related biochemically and serologically to the Salmonella group, they are often mistaken for salmonellae. Such mistakes are not serious, in the authors' opinions, since the organisms produce clinically similar diseases and the same precautions should be taken to prevent transmission of the infections.

In addition to gastroenteritis, Arizona serotypes cause extra-intestinal infections of man. Edwards (10) and Martin (35) observed that Arizona extraintestinal infections appear to represent a higher percentage than one would expect from infections due to non-host adapted Salmonella. These investigators suggested that isolations of Arizona from man probably constitute highly selected material and the high percentage of cases in which these organisms are found extraintestinally is because they are generally not detected in uncomplicated cases of diarrhea. The serotypes 7:1, 2, 6 and 7:1, 7, 8 appeared to be especially invasive in man.

As mentioned previously, Arizona infections of turkeys, chickens, and reptiles have been confirmed (10, 11, 35). Turkey and chicken infections have been transmitted through the medium of eggs.

Absence of Published Arizona Heat Resistance Information

In contrast to the extensive published Salmonella heat resistance data involving various bacteriological media as well as specific food products, which has been recently reviewed (40), a thorough search of the scientific literature has revealed the absence of Arizona heat resistance information.

Sodium Chloride Induced Thermal Resistance

Factors Affecting Thermal Resistance

The thermal resistance of bacteria is influenced by many factors. Among these are: the composition of the culture media in which the organisms are grown, heated, and recovered; the density of the bacterial suspension; the time and temperature of incubation before, during, and after heating. Specific factors such as pH, salt content, availability of water or water activity (a_w) of the media utilized; the technique of exposing bacteria to heat (sealed ampules or open tubes); and the nature of the heat (moist or dry) have all been shown to influence thermal resistance. Such factors have been the subject of many investigations and reviews (3 , 25 , 26).

The effectiveness of a heat treatment is measured by determining the length of time necessary at a specific temperature to kill ninety percent of the organisms originally present (time necessary for the survivors to traverse one log cycle). The time, generally called the D value or decimal reduction time, can be experimentally estimated for a given microorganism by inspection of the exponential death curve at a given temperature or more precisely by calculation. In heat processing studies, thermal death times (TDT), defined as the minimum time necessary at a specific temperature to reduce a given population to below detectable levels, may be determined. For salmonella investigations TDT values are determined with experimental populations that most often have initially 10^6 to 10^8 cells per ml. D values can be determined under a single set of conditions but at different temperatures and used to plot a thermal resistance curve (log D versus temperature in ^oF). The slope of the line measured in ^oF is designated Z, the increase in processing temperature in ^oF required to reduce the D value one log cycle (a ninety percent reduction). The establishment of Z values for a given microorganism allows the calculation of equivalent thermal processing times and temperatures. D values vary considerably among strains and can be greatly influenced by factors mentioned above (40).

Ng, Bayne, and Garibaldi (41) determined the heat resistance of three hundred Salmonella cultures representing seventy-five different serotypes and compared their sensitivities to a reference strain, Salmonella typhimurium Tm-1, a strain of average heat resistance. The D value for S. typhimurium Tm-1 determined at 57° C (134.6 °F) and pH 6.8 in trypticase soy broth - yeast extract (TSB-YE) was 1.2 minutes. The average resistance of almost all strains was 1.2 times that of Tm-1. The least resistant strain was slightly more than half as resistant. One S. blockley strain was five times as resistant as Tm-1. The authors observed that Salmonella cells from the exponential growth phase (physiologically young) are less heat resistant than those in the stationary phase (physiologically old). In keeping with the observations of Elliker and Frazier (12) regarding Escherichia coli heat resistance, Ng et al. reported a direct relationship between heat resistance and the temperature at which the cells tested were grown. Taking such factors into consideration as physiological state, growth temperature, and additional factors mentioned previously, a several hundredfold difference in heat resistance has been demonstrated among the extremes of Salmonella strains. It has been recommended that these recognized differences be taken into consideration in designing effective heat treatments for destroying salmonellae (40).

Water Activity

The amount of water that is available for biological reaction with microbial cells in a given environment has been defined as the water activity (a_w, available water) of that environment (24). All water molecules that are not chemically bound are theoretically available to the cells. The only requisite for a substance to bind water is that the substance be soluble in water. Thus salts, sugars, and proteins will all bind water and remove it from interaction with the microorganism. The water activity is defined as the ratio of the partial pressure of the water vapor above a given solution to the partial pressure of the water vapor above pure water at the same temperature, or

$$a_w = p/p_o$$

where p and p_o are the vapor pressures of the given solution and pure water respectively. The water activity is related to the relative humidity in the following way

$$\frac{\text{Relative Humidity}}{100} = a_w$$

Thus a_w values will fall within a range of 0.0 to 1.0; the former value denoting the complete absence of free water while the latter represents the a_w of pure water (24).

In recent studies of the relation of Salmonella heat resistance to the a_w of the environment, several investigators have reported that, in general, reduction of the a_w of the environment in which salmonellae are heated resulted in an increase in their heat resistance (3 , 7, 25, 38).

On the basis of experiments with eight Salmonella strains in solutions of sucrose, fructose, glycerol, and sorbitol, Goepfert, Iskander, and Amundson(25) reported that there was not a direct correlation between a_w per se and heat resistance of salmonellae: heat resistance did not depend on a_w alone but the nature of the substance that effected the given a_w level was of primary importance. The magnitude of the increase in heat resistance varied from strain to strain. It was possible to place the salmonellae into groups with respect to their heat resistance in sucrose solutions: a "heat-sensitive" group and a "heat-resistant" group. In addition, the response of individual strains to increment decreases in a_w was not uniform. The author observed that this type of behavior made it extremely difficult, if not impossible, to predict heat resistance changes with regard to a_w changes in a given medium. He concluded that the heat resistance of salmonellae in solutions having a reduced a_w could not be inferred from data collected from tests conducted in a single medium but should be determined experimentally on a controlling substance basis. These observations and conclusions pertained only to solutions with an a_w greater than 0.75.

In a study of the survival of Salmonella anatum heated in solutions of various chemical constituents of foods including salts, carbohydrates, amino acids, and peptides, Moats, Dabbah, and Edwards (38) observed that chemically similar substances at the same concentrations showed large differences in protective ability. They concluded, in accord with the conclusion of Goepfert et al. (25), that the chemical nature of the suspending medium is of far more importance than the a_w within the range of a_w greater than 0.75. They offered the explanation that compounds showing a protective effect complex with heat-sensitive protein molecules in the cell, increasing their stability to heat. The lack of protective effect was explained as the inability of molecules to penetrate to heat sensitive sites within the cell.

Similarly, in experiments designed to evaluate the effect of water activity on heat sensitive and heat resistant salmonellae, Baird-Parker, Boothroyd, and Jones (3) determined the resistances of two heat resistant serotypes and two heat sensitive strains of these serotypes in heart infusion broth containing increasing amounts of sucrose, sodium chloride, or glycerol. The observed changes could not be related directly to the a_w of the heating medium but depended more on the solute it contained. The authors concluded that it was impossible to predict the heat resistance of salmonellae in food of low a_w and that in order to find a heat treatment to destroy salmonellae both D and Z values should be determined in that food.

Effect of Sodium Chloride in Various Bacteriological Media

Reviewing factors affecting the heat resistance of nonsporing microorganisms, Hansen and Riemann (26) observed that the effect of salt is variable and depends on the specific salt, the concentration, the suspending medium, and the test organism. They indicated that salts such as sodium and potassium chloride have a pronounced effect on the hydration of proteins and may thereby influence the stability of enzymes and other proteins. Soluble salts may decrease the water activity and thereby increase the resistance of bacterial cells by a mechanism similar to the effect of drying. The authors concluded that it was almost impossible to predict the effect.

Moats *et al.* (38) reported that sodium chloride (NaCl) dissolved in 0.1 M phosphate buffer (pH 7.0) only slightly protected *S. anatum* at the level of 5% NaCl and less at 10% NaCl. Garibaldi *et al.* (23) indicated that NaCl had no protective effect on salmonellae heated in an unspecified buffer system. Baird-Parker *et al.* (3) reported that D values determined in heart infusion broth showed the resistances of heat resistant salmonellae to be generally reduced by the addition of

NaCl although a slight increase in resistance was obtained at the 6.1% NaCl level. In contrast, heat sensitive strains tended to be more resistant in NaCl. Maximum resistance again occurred at the 6.1% NaCl level and resistance decreased with further increase in salt concentration. Cotterill and Glauert (7) found that maximum thermal resistance of Salmonella oranienburg in trypticase soy broth occurred at the 4% NaCl level when held for two days at 6° C before heating and at the 2% NaCl level when not held. Stumbo (44) reported that the heat resistance of many bacteria species was progressively increased by NaCl concentrations up to 4%. Above 4% the heat resistance tended to decrease.

Effect of Sodium Chloride in Liquid Whole Egg and Egg Yolk

In 1967 production of liquid eggs in the United States totaled 801.7 million pounds (46). Of these liquid eggs, 434.9 million pounds were stored in frozen form for future use; 307.3 million pounds were used in liquid form. Ten percent salt or sugar is commonly added to eggs that are to be frozen to prevent gelation during storage. The choice of salt or sugar depends upon the intended use of the eggs. Unpasteurized eggs have been considered safe in the commercial manufacture of mayonnaise and salad dressing of specified compositions because of the sensitivity of salmonellae to the low pH (less than 4.0) of these products at room temperature (40).

Garibaldi, Straka, and Ijichi (23) reported the tenfold increase of Salmonella heat resistance (10 D) in salted or sugared egg yolk over that in whole egg. It was observed that the required USDA treatment of liquid whole egg at 140° F (60° C) for 3.5 minutes as a means of killing salmonellae had been successful for many years. The regulations governing the grading and inspection of egg products (1) specify that all egg products shall be flash-heated to such temperatures and held for such times as will give salmonella killing effects equivalent to the prescribed treatment for liquid whole eggs. Garibaldi et al. indicated that the more severe pasteurizing requirements for yolks are based on the finding that salmonellae are 1.5 to 2.0 times as resistant in yolks as in whole eggs. Recognizing that salt and sugar decrease the flow rate of whole eggs and egg yolks in pasteurizing tubes, and that under commercial conditions the minimum holding time may be 60 to 80% of the average holding time, 146° F (63.5° C) for 3.5 minutes was extrapolated and proposed as the required temperature and time for pasteurizing salted and sugared egg yolk and salted whole eggs (10% salt or sugar added). D values were not determined in salted whole egg but were extrapolated from salted egg yolk data. Survivor curves (the semilogarithmic plot of $\log_{10}$ of surviving cells upon a logarithmic scale against time on a linear scale) of S. typhimurium Tm-1 in yolk, salted, and sugared yolk were logarithmic, i.e., straight lines (21).

Prior to the recommendations of Garabaldi _et al._ (23), Cotterill and Glauert (6) discovered that the addition of salt or sugar increased the temperatures required to destroy _S. oranienburg_ in egg yolk. Salted yolks required a much higher temperature than sugared yolks. The experimental results and recommendations of Cotterill and Glauert (7) in subsequent studies of the effect of salt and sugar on the heat resistance of _S. oranienburg_ in liquid whole egg and egg yolk products have been in striking contrast to those of Garibaldi.

Defining Fm (marginal death temperature) as the minimum temperature, using a 3.75 minute period of heating, required to destroy 99.99% of the cells inoculated into the product (0.01% survival level) Cotterill and Glauert (7) found that the addition of salt (1 through 10%) to liquid whole egg curvilinearly increased the Fm to 67° C, 9.5° C above plain whole egg. Ten percent salt in egg yolk caused the Fm to increase to 68° C, 8.5° C above plain egg yolk. However, in contrast to the linear survivor curves described by Garibaldi (21), these investigators found that the survival curve in yolk containing 10% salt appeared multiorder in nature (non-linear). During the initial period of heating the destruction of the first 90% of organisms was rapid, indicating an initial heat sensitive phase. The subsequent heat resistance of the organisms in the presence of salt increased and the survival curve approached linearity through 99% reduction of the population. The remaining survivors had greater heat resistance than would be expected by linear extrapolation of the survivor curve (tailing effect).

The authors concluded that the multiorder effect was induced specifically by the presence of salt in the media tested. Similar results were obtained with several techniques used to determine heat resistance including thermal death tubes, tubular pasteurizer, and batch pasteurizer. They reasoned that the higher heat resistance in the presence of salt was related to water activity or dehydration of the cell: the degree of cellular plasmolysis would proportionately affect heat resistance. This osmotic effect would be enhanced by elevated but sublethal temperatures during the come-up phase of heating (time period required for the heated medium to reach test temperature). When the come-up phase was eliminated by direct inoculation into the heated product the increased thermal resistance developed at lower survival levels (extension of the heat sensitive phase) since the cells had less opportunity to become heat resistant.

Since most survival curves in salted yolk were non-linear with "tailing-off" of heat resistant cells at low survival levels, it was observed that extrapolation of the survival curves could give erroneous results. F determinations (defined as the time required to destroy all cells at a given temperature) indicated that a heat treatment of 73° C for 3.75 minutes was required to provide adequate pasteurization of salted yolk. However, on the basis of information on heat damage to egg products, lack of salmonella growth in salted yolk, and the primary use of salted yolk in high acid foods, these investigators recommended a pasteurization temperature of 68.0-69.0^{o} C (154.4-156.2^{o} F) for 10% salted egg yolk.

Interpretation of Nonlogarithmic Survivor Curves of Heated Bacteria

The kinetics of thermal death of bacteria have been discussed in detail by several authors, including Hansen and Riemann (26), Vas and Proszt (48), and Moats, Dabbah, and Edwards (37). Moats et al. (36), in a study of nonlogarithmic survival curves of heated bacteria, suggested that the theoretical basis for assuming that thermal death of bacteria is logarithmic, the basis of thermal process calculations used for food processing, stands on two assumptions: (1) populations of single strains of bacteria are homogeneous with regard to heat resistance; and (2) thermal death of bacteria is unimolecular, that is, death occurs from inactivation of a single molecule or critical site per cell. Experimental evidence presented by these investigators, however, indicated that assumption (1) is seldom true and that assumption (2) is incompatible with the multiple critical site hypothesis of thermal death.

Complex survivor curves were interpreted to be composites of several convex survivor curves that represent populations of different heat resistances in a single culture of bacteria. Changes in overall heat resistance of a culture, depending on the age of the culture, indicated that differences in heat resistances of bacterial cells in a homogeneous culture were determined physiologically. The shapes of the curves also changed with the age of the culture, suggesting that the relative proportion of cells of different heat resistances also changed. Bacteria in the stationary growth phase sometimes gave simple convex survivor curves and were interpreted as indicating a population homogeneous with regard to heat resistance. Logarithmic survival curves were explained as the result of a fortuitous mixture of cells of different heat resistances.

The authors concluded that the heat resistance of a genetically homogeneous bacterial population may follow a regular distribution, an irregular distribution, or may be homogeneous, depending on physiological factors which are not readily controlled. They noted that: flat heat resistant tails have been reported by many investigators but they have failed to determine how resistant the few survivors were; investigators who start with low initial populations or carry survival curves through only four or five log cycles would completely miss small heat resistant populations. In addition, if thermal death of bacteria is not truly logarithmic, they reasoned, decimal reduction times (D values) are meaningless. Extrapolation of D values from survivor curves carried through four or five log cycles would give seriously misleading results. Consequently, F values were proposed as a replacement for D values. F value, the time in minutes for a given bacterial population to be reduced by a given percentage ($F_{99.9}$, $F_{99.99}$, etc.) under specific commercial conditions, would take tailing of survivor curves into consideration.

The multiple critical sites theory of thermal death proposed by Moats (36) explains thermal death of bacteria by assuming that death results from inactivation of a small fraction of a number of critical sites (probably greater than 100). This hypothesis also assumes that the bacterial population is homogeneous in heat resistance. The nature of the critical site is unknown. The author maintains that the critical sites could be structures in or making up the cell membrane. Damage to the cell membrane could allow penetration of toxic agents which could not pass through the intact membrane. The multiple critical sites theory cannot predict the occurrence of extensive tailing (8) but nonlogarithmic survivor curves have been cited as a reflection of this proposed mechanism of thermal death (37).

MATERIALS AND METHODS

Media

The medium used in preliminary screening and more detailed experimentation in bacteriological broth was Trypticase Soy Broth (BBL) supplemented with 2.0% (w/v) yeast extract (Nutritional Biochemicals Company; TSB-YE) or Trypticase Soy Agar similarly supplemented (TSA-YE). The pH of these media was adjusted to 7.0 with 1.0 N NaOH and autoclaved, yielding a final pH of 6.8. AR grade NaCl was added to TSB-YE to give final concentrations of 4.0 and 10.0% NaCl (w/v) and the final pH similarly adjusted to 6.8. TSB-YE without added NaCl contains 0.5% NaCl. In addition to TSB-YE, 0.1% (w/v) Bacto-peptone (Difco) was used as dilution medium with a final pH of 6.8.

Antibiotic free eggs approximately one to two weeks old were immersed for five minutes in 70% ethyl alcohol to kill microorganisms on the surface and then allowed to air dry at room temperature. They were broken out and aseptically added to a sterile blender jar and blended in a commercial Waring blender for two minutes. Sterile AR grade NaCl was added to the plain whole eggs to yield a final liquid product containing 10.0% NaCl (w/w) when similarly blended for two minutes. Plain and salted liquid whole egg had a pH of 7.0 - 7.2.

Bacterial Strains

Several Salmonella and Arizona cultures were obtained by OSDA laboratory isolations from liquid whole eggs and mayonnaise. The Salmonella typhimurium Tm-1 culture, a reference strain with heat resistance characteristics that are comparable to those of other salmonellae (41), was provided by the USDA Western Regional Research Laboratory, Albany, California. The Arizona neotype culture (13314) was obtained from the American Type Culture Collection, Rockville, Maryland. These cultures were maintained and transferred weekly as slant cultures on TSA (BBL) slants.

Growth of Cultures

Cultures were grown in 250-ml Erlenmeyer flasks containing 50 ml TSB-YE at 35^{o} C with an incubated shaker. The cell suspensions were prepared from cultures well into the stationary growth phase, which provides cells of maximum heat resistance (41), by using 24 $\pm$ 1 hour cultures.

Viable Cell Counts

The number of viable cells (survivors) was determined by appropriately diluting samples in 0.1% peptone or TSB-YE and spread or pour plating onto TSA-YE plates in duplicate, depending on the method used to determine heat resistance. The plates were incubated at 35° C for 24 hours since longer incubation periods did not increase the plate counts but allowed colony size to interfere with accurate counting.

Preliminary Screening for Thermal Resistance

The relatively rapid procedure devised to screen a large number of cultures for heat resistance by Ng *et al.* (41), was used in this study. It consisted of diluting 1 ml of each culture grown at 35° C in TSB-YE into 99 ml of ice-cold TSB-YE and dispensing 2 ml of the diluted cells into each of two 13- by 100-mm screw cap test tubes. One tube of each culture was kept on ice, whereas the other was immersed up to the cap in a bath at 57 ± 0.1 C for 10.0 minutes. After cooling the heated tubes in an ice bath, the viable cell count was determined on the contents of both the heated and unheated tubes. DRT (D) values were calculated from this single time period and were compared. Plotting the log of survivors against time allows the estimation of the D value, which is the time required to reduce the population by 90%. Using this method the heat resistances of several *Salmonella* and *Arizona* laboratory isolated strains were estimated in TSB-YE with 0.5, 4.0, and 10.0% NaCl.

Characterization of NaCl Induced Heat Resistance in TSB-YE and Liquid Whole Egg

A procedure derived from the methods used by Garibaldi *et al.* (23) to study the heat resistance of *S. typhimurium* Tm-1 in various egg products was applied to the present study. Iced TSB-YE or liquid whole eggs were innoculated with the cell suspensions described above to give a final population of approximately 10^7 per ml. Two-ml amounts of the inoculated media were dispensed with 2.0 ml hypodermic syringes fitted with 1 1/2 in. 21 guage needles into 2-ml color-break ampules (Kimble), which were then sealed and cooled to $\sim 0.0^{\circ}$ C in an ice-water bath for approximately one hour. The sealed ampules were removed from the ice-water bath, shaken for five seconds with a vortex mixer (Scientific Products), and completely immersed in a heated water bath with temperature control of $\pm 0.1^{\circ}$C. After various periods of exposure duplicate ampules were removed from the heated water bath and the contents were cooled immediately in an ice-water bath with rapid shaking. Ampules were left in the ice-water bath for at least one hour and, when removed, were shaken for five seconds with a vortex shaker immediately before diluting. Appropriate 0.1% peptone dilutions of the samples were spread-plated in duplicate on TSA-YE plates in the TSB-YE studies but pour-plated in the liquid whole egg studies in order to analyze the sample without dilution. It was experimentally determined that the plating method did not effect the number of survivors. After incubation at 35^{o}C for 24 hours colonies were counted.

Data Analysis

Survivor curves, constructed by plotting the logarithim of the number of survivors against the period of exposure to heat were used to determine D and F values. The slope of the line over a time interval necessary to result in at least a 10^5 kill was used to determine all D values except those given in the preliminary screening previously described. F values, defined as the time in minutes required to reduce a population by a given percentage (99.0, 99.9, etc.), under specified conditions, were also determined. Kinetic data of the type determined in this study, actually measure a degree of injury as shown by the loss of ability to reproduce on a given medium. This gives a reproducible end point and is therefore a valid method for kinetic studies (36).

RESULTS

Preliminary Screening of Salmonella and Arizona Sodium Chloride Induced Heat Resistance

The D_{57^o} values obtained from preliminary screening of laboratory isolated strains of Salmonella and Arizona are presented in Table 2. D_{57^o} values obtained in TSB-YE at 0.5, 4.0, and 10.0% NaCl levels increased progressively. Salmonellae and arizonae had similar heat resistance at the 0.5% NaCl level with D_{57^o} values that varied from 2.5 to 3.2 minutes. The heat resistance of all strains increased at the 4.0% NaCl concentration with D_{57^o} values ranging from 6.0 to 10.0 minutes: the two Arizona 7 : 1, 7, 8 strains had greater heat resistance than either S. reading or S. san diego. In contrast to previous reports (3 , 44), each individual strain had greater heat resistance with 10.0% NaCl than with 4.0% NaCl: D_{57^o} values with 10.0% NaCl varied from 9.5 to 17.0 minutes. Arizona 7 : 1, 7, 8 (OSDA 1195) had the greatest heat resistance at both the 4.0 and 10.0% NaCl levels while Arizona 7 : 1, 7, 8 (OSDA 3840) had an intermediate D_{57^o} value with 10.0% NaCl. Experiments were initiated to determine in greater detail the relative heat resistance of the Salmonella and Arizona groups in TSB-YE with and without added salt.

Characteristics of Sodium Chloride Induced Heat Resistance in TSB-YE

Three Salmonella - Arizona strains were used to characterize NaCl induced heat resistance in TSB-YE: S. typhimurium Tm-1

Table 2. D_{57^o} Values of *Salmonella* and *Arizona* Serotypes: Preliminary Screening Results in TSB-YE with 0.5, 4.0, 10.0% NaCl.

Serotype	D_{57^o} values (minutes)		
	0.5% NaCl	4.0% NaCl	10.0% NaCl
Arizona 7 : 1,7,8 (OSDA 1195)	2.5	10.0	17.0
Arizona 7 : 1,7,8 (OSDA 3840)	2.9	8.7	11.5
Salmonella reading (OSDA 883)	2.5	6.0	13.5
Salmonella san diego (OSDA 3841)	3.2	7.1	9.5

(USDA), a reference strain reported to have heat resistance characteristics comparable to those of other salmonellae (41); the *Arizona neotype* strain (ATCC 13314); and an *Arizona 7 : 1, 7, 8* strain (OSDA 3840) that had intermediate heat resistance in preliminary testing. Typical survivor curves for *S. typhimurium* Tm-1 and the *Arizona neotype* strain are presented in Figures 1 and 2, respectively. Survivor curves for *Arizona 7 : 1, 7, 8* (3840) were similar to *S. typhimurium* Tm-1 curves.

The survivor curves represented in Figures 1 and 2 clearly demonstrate NaCl induction of nonlogarithmic thermal death kinetics in TSB-YE with 4.0 and 10.0% NaCl, as opposed to nearly logarithmic death kinetics in 0.5% NaCl. It has been reported that nonlogarithmic, multiordered thermal death kinetics invalidate the use of D values to calculate heat resistance (8, 36, 37). Therefore, D_{57^o} values were calculated for 0.5% NaCl only. $F_{99.9999}$ (6-log kill) values were determined for all three salt concentrations (Table 3).

Table 3. The Effect of NaCl on F_{57^o} Values of *Salmonella* and *Arizona* Serotypes in TSB-YE.

Serotype	D_{57^o} values (minutes) 0.5% NaCl	F_{57^o} (99.9999) values (minutes) 0.5% NaCl	4.0% NaCl	10.0% NaCl
S. typhimurium Tm-1 (USDA)	3.0	17.0	80.0	75..0
Arizona neotype (ATCC 13314)	1.5	12.0	80.0	110.0
Arizona 7 : 1, 7, 8 (OSDA 3840)	1.1	7.0	80.0	75.0

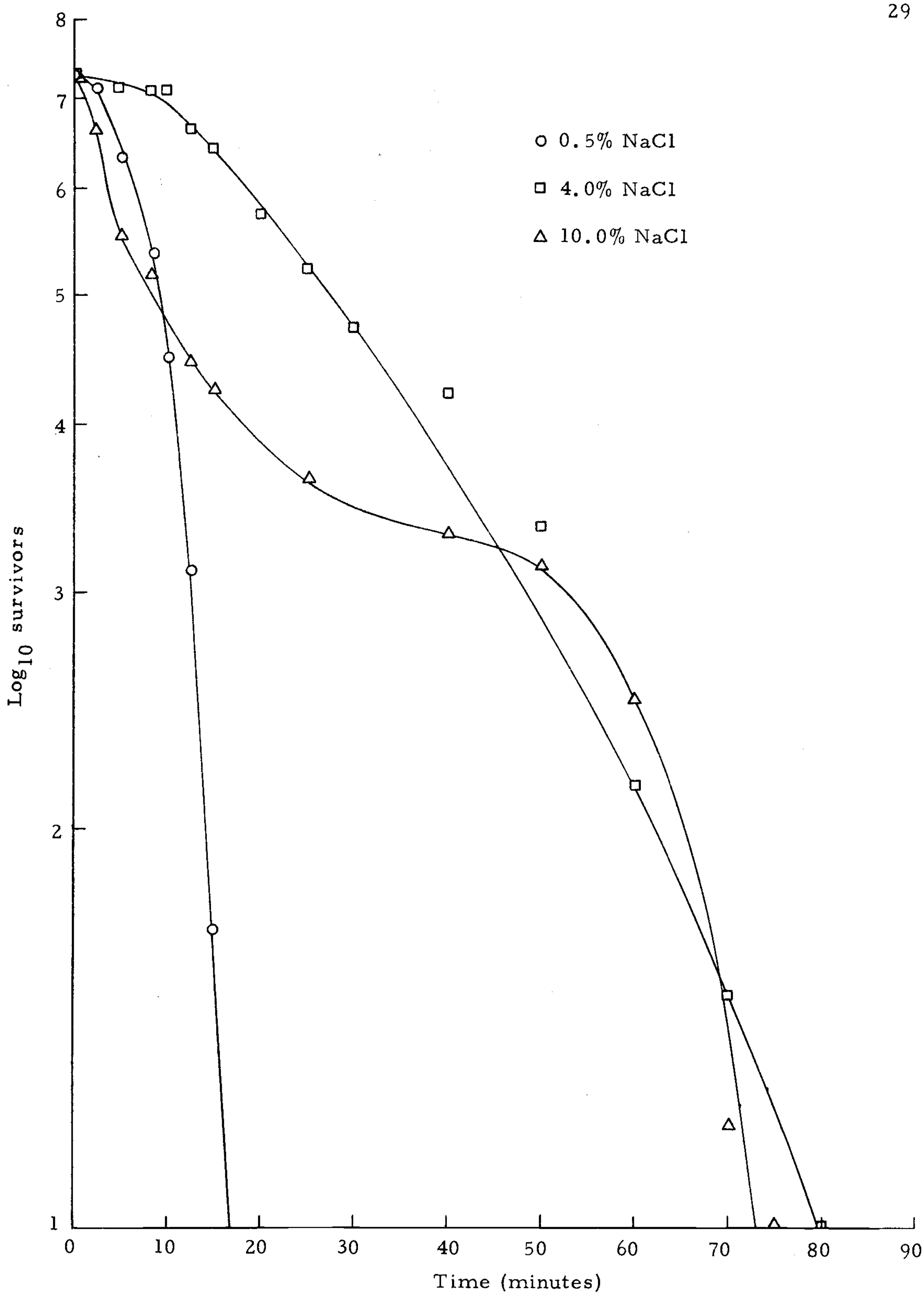

Figure 1. Survival Curves of S. typhimurium Tm-1 in TSB-YE with 0.5, 4.0, and 10.0% NaCl at 57° C.

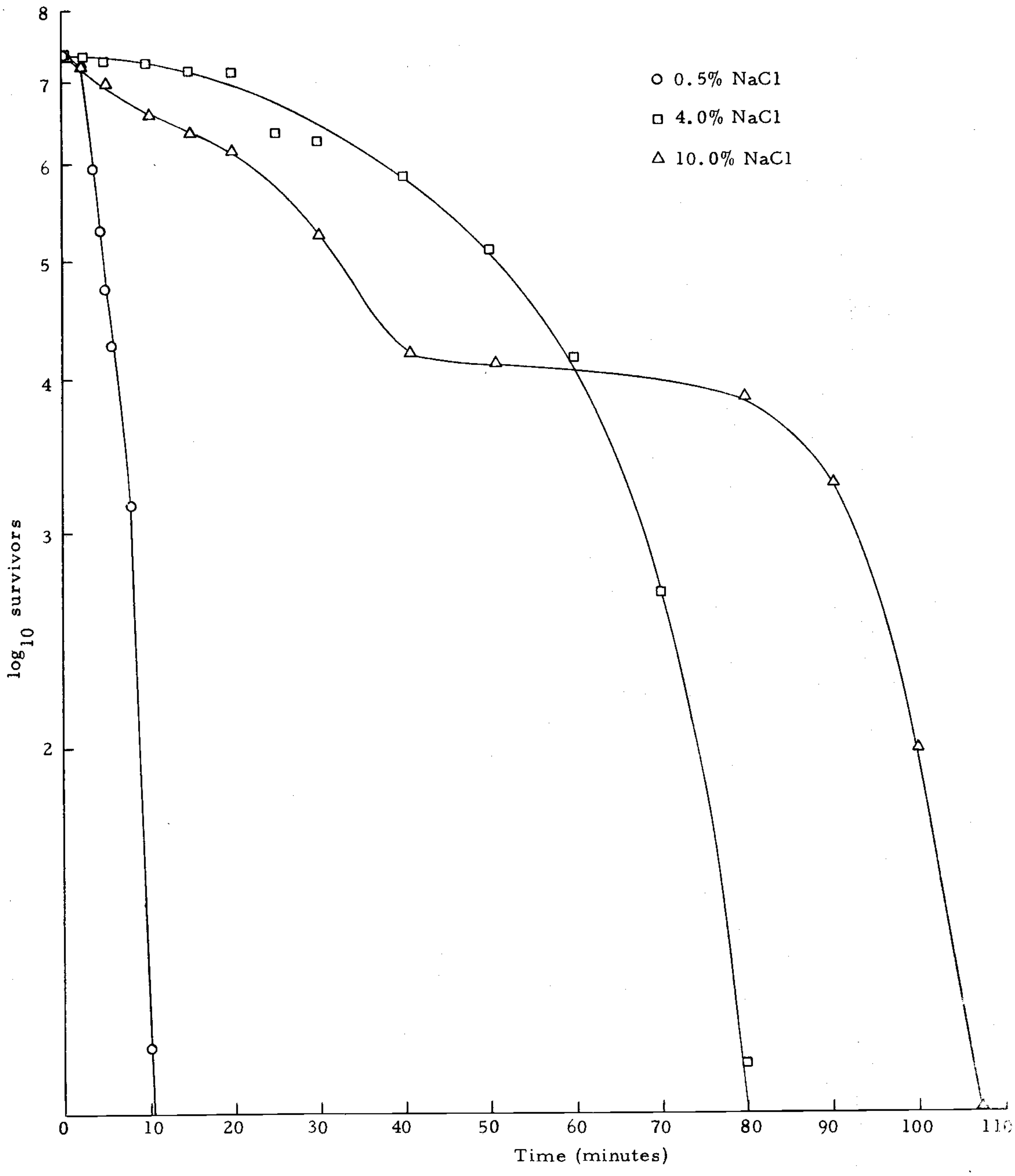

Figure 2. Survival Curves of Arizona neotype (13314) in TSB-YE with 0.5, 4.0, and 10.0% NaCl at 57°C

D_{57^o} and F_{57^o} values for 0.5% NaCl showed that S. typhimurium Tm-1 has greater heat resistance in TSB-YE without added salt than either Arizona strain. All three strains had greater heat resistance in 4.0% NaCl than 0.5% NaCl; only Arizona neotype had greater heat resistance in 10% NaCl than 4.0% NaCl. F_{57^o} values for 4.0% NaCl indicate that all three strains survived the heat treatment for 80 minutes. F_{57^o} values for 10% NaCl show the Arizona neotype to have greater heat resistance than S. typhimurium Tm-1 and Arizona 7 : 1,7,8 (3840) which again had similar heat resistance but less than at the 4.0% NaCl level.

The survival curves developed in 4.0 and 10% NaCl (Figures 1, 2) reveal significant differences among the strains tested. In TSB-YE with 0.5 and 4.0% NaCl each serotype developed a simple convex shaped survival curve. Such curves have been interpreted as representative of the thermal death of a bacterial population that is homogeneous in heat resistance (37). The similarity of survival curve shapes and F_{57^o} values developed in 4.0% NaCl indicate similar heat resistance characteristics of salmonellae and arizonae in the presence of 4.0% NaCl.

A comparison of S. typhimurium Tm-1 and Arizona neotype survivor curves in TSB-YE with 10.0% NaCl is presented in Figure 3.

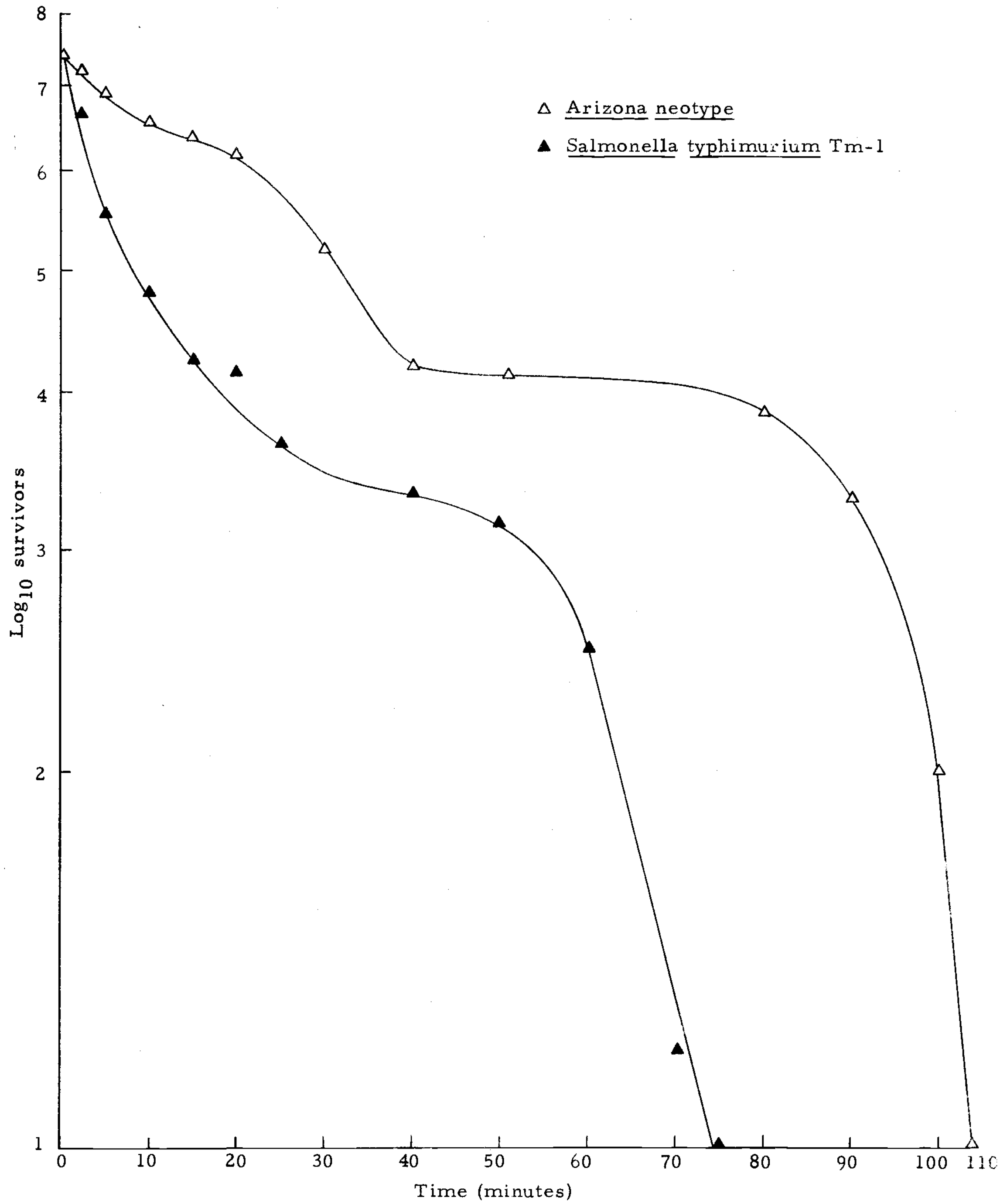

Figure 3. Comparison of S. typhimurium Tm-1 and Arizona neotype (13314) Survival Curves in TSB-YE with 10.0% NaCl at 57° C

Ten percent NaCl, in contrast with 4.0% NaCl, induces a heat sensitive population during the initial phase of heating S. typhimurium Tm-1 and Arizona 7 : 1,7,8 (3840) suspensions. The initial death rate of these serotypes is greater in 10.0% NaCl than in 0.5% or 4.0% NaCl indicating a specific "toxic" effect due to 10.0% NaCl. The survival curve appears multiorder in nature with the initially rapid death rate followed by "flattening" and "tailing" into a more heat resistant population. Such curves have been interpreted as representative of a bacterial population that is heterogeneous in heat resistance (irregular distribution of heat resistance) (37). Thus, the specific effect of 10.0% NaCl in TSB-YE appears to be the induction of distinct "heat sensitive" and "heat resistant" populations within S. typhimurium Tm-1 and Arizona 7 : 1,7,8 (3840) suspensions.

Ten percent NaCl does not induce a "heat sensitive" population during the initial phase of heating Arizona neotype suspensions in TSB-YE. However, a curvilinear survival curve is produced, demonstrating distinct heat resistance differences within the suspension. The failure of 10.0% NaCl to induce "heat sensitive" populations initially in Arizona neotype TSB-YE suspensions may be related to the greater heat resistance, reflected in F_{570} determinations (survival times), when compared to the other serotypes. Thus, 10.0% NaCl induces multiordered thermal death kinetics with the three serotypes examined in TSB-YE.

Characteristics of Sodium Chloride Induced Heat Resistance in Liquid Whole Egg

To compare the effects of 10.0% NaCl in liquid whole egg and TSB-YE, the heat resistances of the three serotypes selected for this study were determined in liquid whole egg with 0% and 10.0% NaCl at 57° C (134.6° F). Typical survivor curves for the three test strains are presented in Figures 4 and 5.

Survival curves of S. typhimurium Tm-1, Arizona neotype, and Arizona 7 : 1,7,8 (3840) in liquid whole egg with 0% added salt (Figure 4) are logarithmic. The initial lag in death rate during the first two minutes of heating is a reflection of the come-up phase of heating the bacterial suspensions. This lag period is not as evident in TSB-YE : 0.5% NaCl survival curves. Survival curves of the three serotypes in liquid whole egg with 10.0% NaCl (Figure 5) demonstrate an initial rapid death rate followed by a reduced death rate with what appears to be logarithmic thermal death kinetics. Close examination of the survivor curves, particularly those for the Arizona neotype, reveals possible shoulders in the curves resembling the curvilinear survivor curves obtained in TSB-YE : 10.0% NaCl, indicating the transition with time of heat exposure to populations of different heat resistances within the bacterial suspensions. However, 10.0% NaCl in liquid whole egg induces "heat sensitive" populations during the initial heating phase of Arizona neotype as well as S. typhimurium

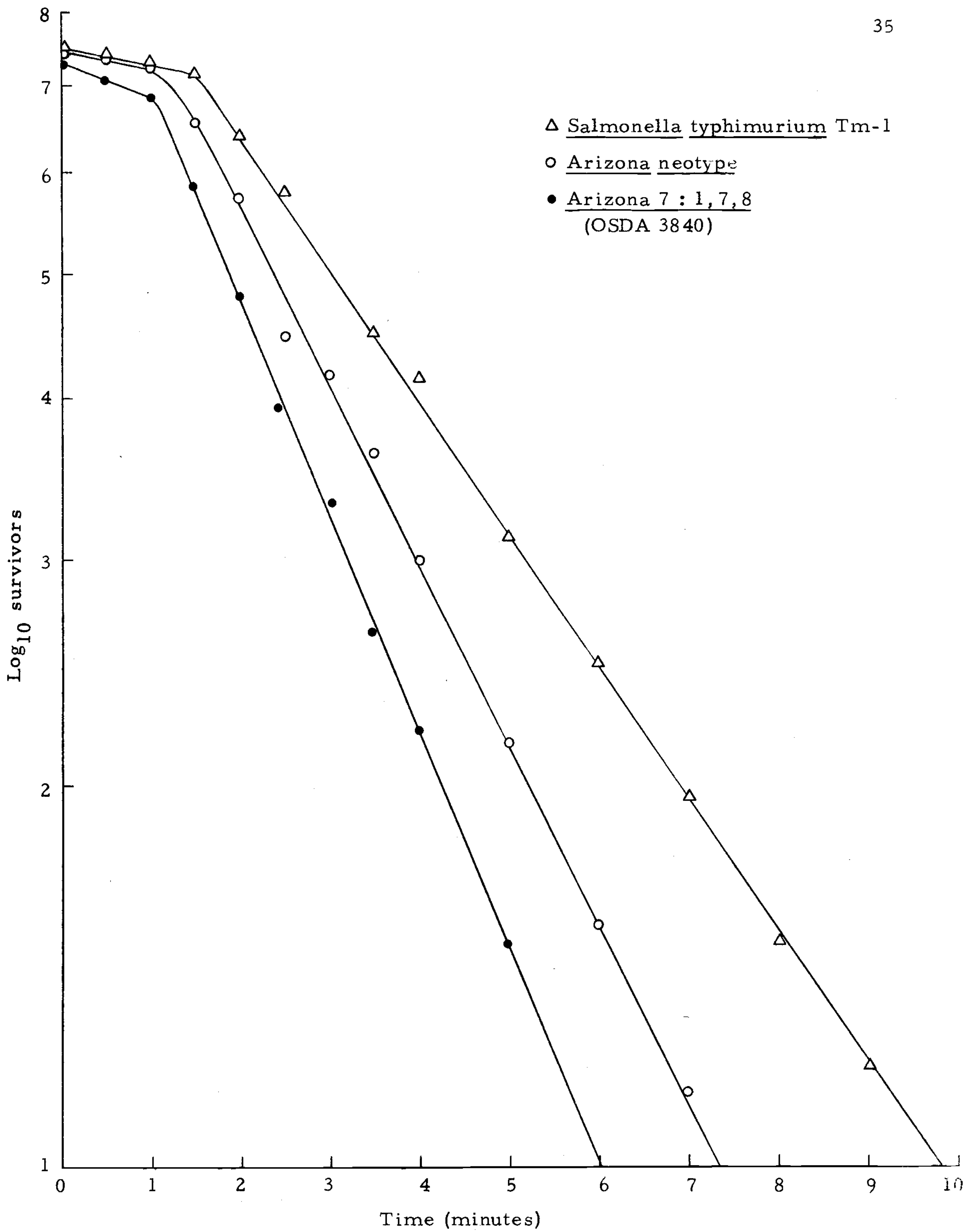

Figure 4. Survival Curves of S. typhimurium Tm-1, Arizona neotype (13314), and Arizona 7 : 1, 7, 8 (3840) in Liquid Whole Egg Without Added NaCl at 57° C.

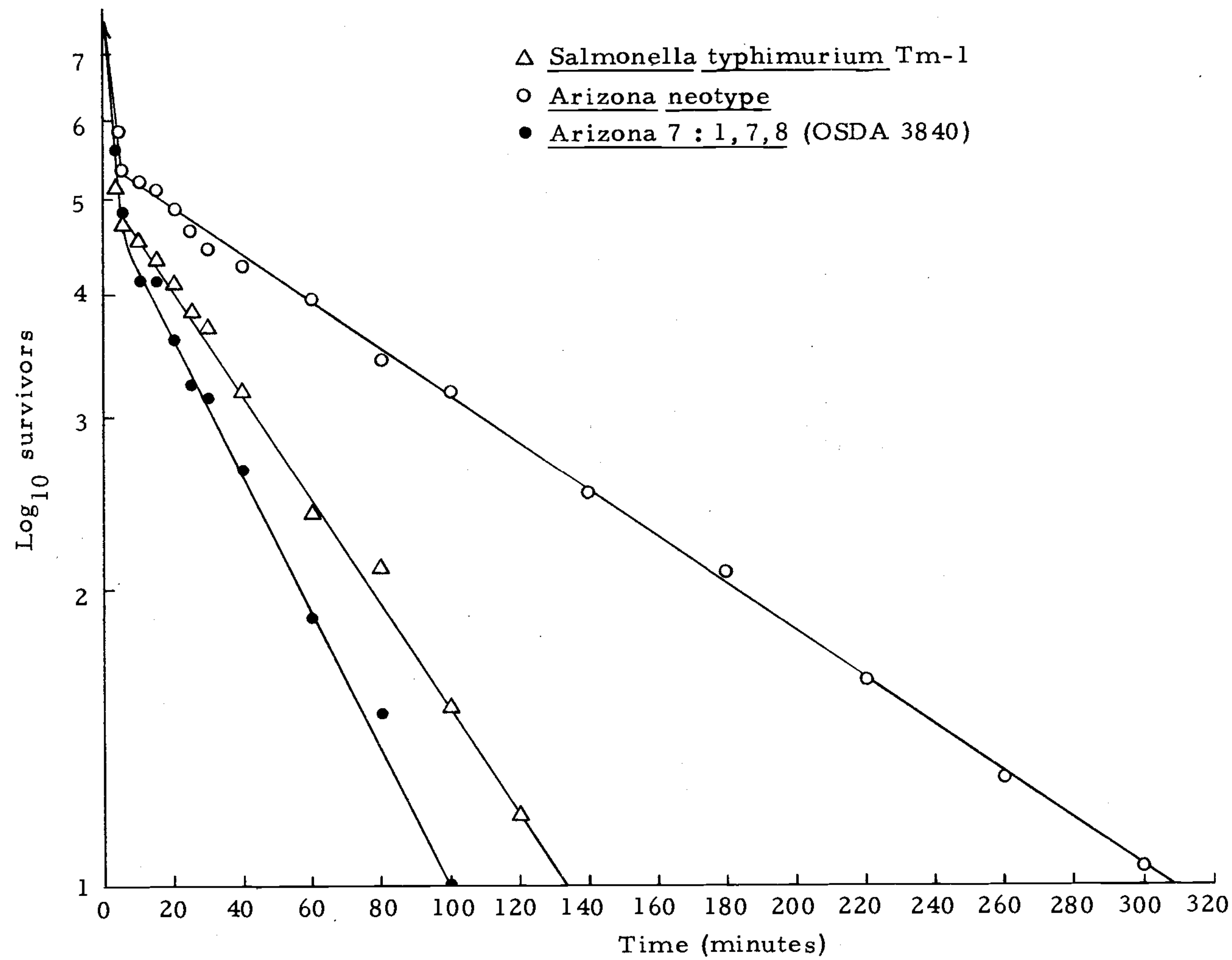

Figure 5. Survival Curves of S. typhimurium Tm-1, Arizona neotype (13314), and Arizona 7 : 1,7,8 (3840) in Liquid Whole Egg with 10.0% NaCl at 57° C.

Tm-1 and Arizona 7 : 1,7,8 (3840) suspensions. The initial death of the three test strains in liquid whole egg is greater in 10.0% NaCl than in 0% NaCl indicating a toxic effect of 10.0% NaCl. Thus, the specific effect of 10.0% NaCl in liquid whole egg at 57° C is induction of distinct "heat sensitive" and relatively more "heat resistant" populations within test strain suspensions. In contrast to TSB-YE : 10.0% NaCl, which induced obvious curvilinear - multiordered death kinetics, 10.0% NaCl in liquid whole egg induced apparent logarithmic thermal death kinetics within the "heat sensitive" and "heat resistant" populations of each bacterial suspension.

D_{57^o} and F_{57^o} determinations are presented in Table 4. S. typhimurium Tm-1 has greater heat resistance, as revealed by D_{57^o} and F_{57^o} values, in plain liquid whole egg than either Arizona strain. Thus, the order of heat resistance among the test strains is the same as occurred in TSB-YE with 0.5% NaCl.

Ten percent NaCl induces greater heat resistance of all three test strains in liquid whole egg than in TSB-YE. The Arizona neotype developed the highest D_{57^o} and F_{57^o} values in liquid whole egg with 10.0% NaCl, followed by S. typhimurium Tm-1 and Arizona 7 : 1,7,8 (3840). In contrast to previous reports (23) 10.0% NaCl produced more than a 10 D increase in heat resistance in liquid whole egg: there was a 25 D increase in heat resistance of Arizona neotype when compared to the plain liquid whole egg D value, followed by 15 D and

Table 4. The Effect of NaCl on D_{570} and F_{570} Values of *Salmonella* and *Arizona* Serotypes in Liquid Whole Egg.

Serotype	D_{570} Values (minutes)		F_{570} Values (minutes)			
	0.0% NaCl	10.0% NaCl	0.0% NaCl*	10.0% NaCl		
			$F_{99.9999}$	$F_{99.9999}$	$F_{99.99}$	$F_{99.0}$
S. typhimurium Tm-1	1.2	18.0	9.8	135.0	50.0	4.5
Arizona neotype	1.0	25.0	7.3	310.0	95.0	7.5
Arizona 7 : 1,7,8	1.0	14.5	6.0	100.0	25.0	4.5

*F_{570} (90.0) values in liquid whole egg: 0% NaCl were < 2.0 minutes for all test strains.

14.5 D increases in S. typhimurium Tm-1 and Arizona 7:1, 7, 8 (3840) heat resistances, respectively. F_{57^o} values reveal that during the first 99.0% reduction ($F_{99.0}$) of the test strain populations, Arizona neotype has approximately twice the survival time (7.5 minutes) of S. typhimurium Tm-1 and Arizona 7:1, 7, 8 (4.5 minutes). The following 99.0% reduction ($F_{99.99}$) of the test strain populations show that the Arizona neotype has nearly twice the survival time (95.0 minutes) of S. typhimurium Tm-1 (50.0 minutes) and four times the survival time of Arizona 7:1, 7, 8 (3840) (25.0 minutes). The last 99.0% reduction ($F_{99.9999}$), resulting from bacterial death traversing six log cycles, indicates that the total survival time of the Arizona neotype suspension (310.0 minutes) is roughly three times that of S. typhimurium Tm-1 (135.0 minutes) and Arizona 7:1, 7, 8 (100.0 minutes).

To determine the effect of NaCl induced heat resistance on the survival characteristics of the Salmonella and Arizona test strains in liquid whole egg during the pasteruizing process, survival curves were constructed for liquid whole egg containing 10.0% NaCl heated to 63.5°C (146.0° F). Typical survival curves, presented in Figure 6, indicate close similarity of heat resistance characteristics of S. typhimurium Tm-1 and Arizona 7:1, 7, 8 (3840): there is no lag period in death but an initial rapid death rate is followed by a reduced death rate that eventually "tails" into a small population of extremely resistant organisms. Extrapolation of these survivor curves through

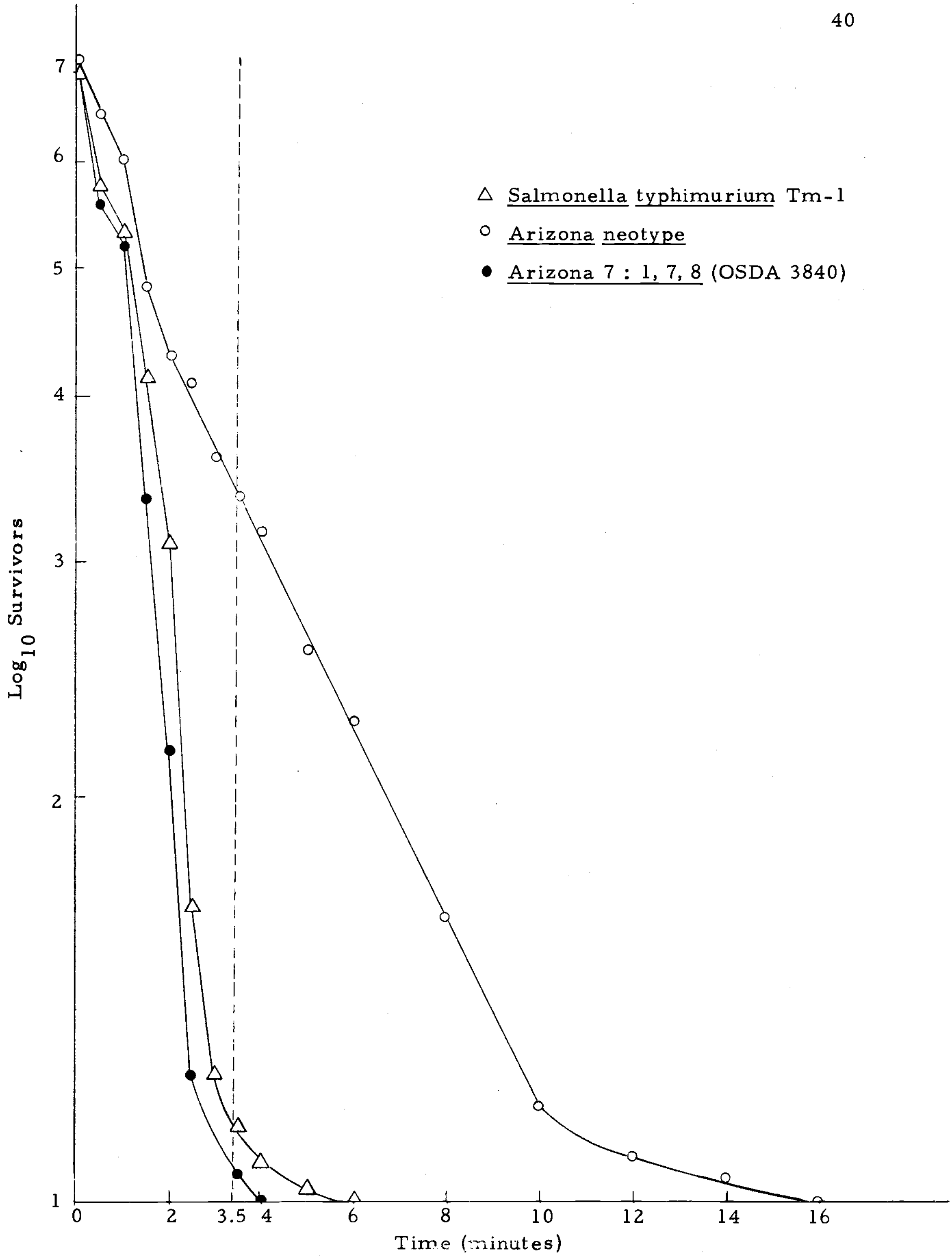

Figure 6. Survival Curves of S. typhimurium Tm-1, Arizona neotype (13314), and Arizona 7 : 1, 7, 8 (3840) in Liquid Whole Egg with 10.0% NaCl at 63.5° C

the initial five log cycles of bacterial death would miss these "tails" and erroneously indicate that the test strains would not survive the prescribed pasteurization treatment of 63.5 °C for 3.5 minutes. The _Arizona neotype_ survivor curve is similar in that there is no initial lag period in bacterial death, but dissimilar in that the heat resistance is considerably greater and, consequently, the initial death rate is not as great as that of the other test strains. As with 57° C determinations, 10.0% NaCl in liquid whole egg induces distinct populations with regard to heat resistance differences at 63.5° C. In contrast to the apparent logarithmic order of death of most of the test strain populations, the "tails" or segments of the survival curves that represent extremely heat resistant populations are curvilinear or multi-ordered.

$D_{63.5^o}$ and $F_{63.5^o}$ values, presented in Table 5, show that 10.0% NaCl induces the same relative order of heat resistances among the test strains at 63.5° C that occurred in liquid whole egg and TSB-YE at 57° C: _Arizona neotype_ has more than twice the heat resistance of the other test strains at 63.5° C as measured by $D_{63.5^o}$ and $F_{63.5^o}$ values. $D_{63.5^o}$ values were determined because they are commonly used in food processing thermal calculations (40) and the prescribed pasteurization treatment of 63.5° C, 3.5 minutes, for salted egg yolk and liquid whole egg is based on extrapolated survivor curves. $F_{63.5^o\ (99.9999)}$ values indicate that all three test strains survive the required pasteurizing time and temperature of exposure.

Table 5. $D_{63.5^o}$ and $F_{63.5^o}$ Values of *Salmonella* and *Arizona* Serotypes in Liquid Whole Egg with 10.0% NaCl.

Serotype	$D_{63.5^o}$ Values (minutes)	$F_{63.5^o\ (99.9999)}$ Values (minutes
Arizona neotype (ATCC 13314)	1.6	16.0
S. typhimurium Tm-1 (USDA)	0.6	6.0
Arizona 7 : 1,7,8 (OSDA 3840)	0.4	4.0

The heat resistance data in Table 5 can be used to derive a simple relationship between $D_{63.5^o}$ values, calculated on the basis of five log cycles of bacterial death, and $F_{63.5^o\ (99.9999)}$ values, calculated on the basis of six log cycles of bacterial death:

$$F_{63.5^o\ (99.9999)} = 10\ D,$$

that is, the survival time of the salmonella and arizonae test strains in liquid whole egg containing 10.0% NaCl at 63.5°C appears to be related to the organism's D value by a factor of 10.

DISCUSSION

Characteristics of Sodium Chloride Induced Heat Resistance in TSB-YE

The results of the present study indicate that the *Salmonella* and *Arizona* test strains have similar heat resistance characteristics in TSB-YE without added NaCl. The addition of NaCl to TSB-YE has a protective effect in that survival times of the test organisms are increased in the presence of NaCl (Tables 2, 3); this confirms the observations of previous workers (7, 44). Preliminary screening of *Salmonella* and *Arizona* laboratory isolates indicates that 10.0% NaCl has a greater protective effect than 4.0% NaCl. Four percent NaCl was chosen for this study because previous reports indicate that maximum bacterial heat resistance occurs near this NaCl concentration (44). Preliminary screening revealed that *Arizona 7 : 1,7,8* (1195) has greater heat resistance in 4.0 and 10.0% NaCl than the other laboratory isolated strains. As a group, *Arizona* laboratory isolates have greater heat resistance than *Salmonella* laboratory isolates in 4.0% NaCl when analyzed by the simplified screening method, but similar heat resistance when survival time is measured with the sealed ampule technique. The difference in results is probably due to the methods of heating the bacterial suspensions; the screening method is considered approximate (41). The heat resistances of the laboratory isolates in TSB-YE without added salt are about 2-3 times the reported heat resistance of *S. typhimurium* Tm-1 when determined by the screening method.

More detailed analysis of *Salmonella* and *Arizona* heat resistance using the sealed ampule technique with two reference strains, *S. typhimurium* Tm-1 and the *Arizona neotype*, and a laboratory isolate, *Arizona 7 : 1, 7, 8* (3840) indicates that *S. typhimurium* Tm-1 has 2-3 times the heat resistance of the *Arizona* strains in TSB-YE with 0.5% NaCl. The similarity of test strain heat resistances in 4.0% NaCl, reduced heat resistance of *S. typhimurium* Tm-1 and *Arizona 7 : 1, 7, 8* (3840) in 10.0% NaCl, and increased heat resistance of *Arizona neotype* in 10.0% NaCl, support the conclusions of Geopfert *et al.* (25), Moats *et al.* (38), and Baird-Parker *et al.* (3) regarding salmonellae heat resistance: there is no direct correlation between a_w per se and heat resistance, rather, the substance that is effecting the reduced a_w is of primary concern. Thus, the arizonae appear to be heterogeneous in response to 10.0% NaCl. It remains to be determined if the *Arizona neotype* response to 10.0% NaCl is characteristic of the *Arizona* or *Salmonella* when so few organisms have been examined.

The survivor curves of the test strains in TSB-YE with 0.5, 4.0, and 10.0% NaCl (Figures 1, 2, 3) indicate that NaCl induces nonlogarithmic, multiordered, thermal death kinetics, as discussed by

Moats (36), and Moats et al. (37). The induction of nonlogarithmic thermal death of salmonellae by NaCl in egg yolk has been reported by Cotterill and Glauert (7). The present study demonstrates that the survival curves of the three test strains in 0.5 and 4.0% NaCl have a simple convex shape, representing a homogenous population (regular distribution) with regard to heat resistance (37). The induction of "heat sensitive" and "heat resistant" populations by 10.0% NaCl in S. typhimurium Tm-1 and Arizona 7 : 1, 7, 8 (3840) suspensions is similar to the toxic effect of NaCl in egg yolk previously reported (7). The results of the present study do not explain the failure of 10.0% NaCl to induce a "heat sensitive" population in TSB-YE suspensions of the Arizona neotype. It is evident, however, that 10.0% NaCl induces heterogeneous populations (irregular distribution) with regard to heat resistance within suspensions of all three test strains (37).

The results of the TSB-YE study clearly indicate that the use of D values to characterize heat resistance induced by NaCl, and possibly reduced a_w in general, as is commonly reported (3, 25, 38), could lead to serious miscalculations of salmonellae and arizonae sensitivity to heat. These observations, in accord with those of Moats (36), Moats et al. (37), and Dabbah et al. (8), indicate that the use of F values appears to be a more accurate measure of an organism's heat resistance under specified conditions. Figures 1, 2, and 3 illustrate that extrapolation of survival curves after only four or five log cycles of bacterial death would lead to incorrect conclusions regarding the survival characteristics of the test organisms.

Characteristics of Sodium Chloride Induced Heat Resistance in Liquid Whole Egg

The data obtained from heat resistance experiments with *S. typhimurium* Tm-1, *Arizona neotype*, and *Arizona 7 : 1,7,8* (3840) in plain (0.0% added NaCl) and salted (10.0% NaCl) liquid whole egg permit several important observations. First, 10% NaCl induces multiordered thermal death kinetics of salmonella and arizona in liquid whole egg at 57°C and 63.5°C closely resembling the salmonella thermal death kinetics induced by 10.0% NaCl in liquid egg yolk (57°C, 59°C) reported by Cotterill and Glauert (7), and in TSB-YE (57°C) reported in this study. Results of this study reveal that all of the test strains have less heat resistance in plain liquid whole egg than in TSB-YE with 0.5% NaCl but 10% NaCl induces greater heat resistance of all test strains in liquid whole egg than in TSB-YE. *S. typhimurium* Tm-1 has the greatest heat resistance in liquid whole egg and TSB-YE without added salt while *Arizona neotype* has significantly greater 10.0% NaCl induced heat resistance in both these media. These observations indicate that NaCl induced heat resistance of the *Salmonella* and *Arizona* strains tested is dependent on the specific composition of the heating medium, as well as the test strain. The role of viscosity can be negated since the addition of 10% NaCl to liquid whole egg and TSB does not change the viscosity of these media (7). The role of a_w of these media can only be a matter of speculation since a_w values were not determined experimentally in this study. However, the ability of 4.0% NaCl to induce greater heat resistance of S. typhimurium Tm-1 and Arizona 7 : 1,7,8 (3840) in TSB-YE than 10.0% NaCl, supports the view that NaCl induced heat resistance is not simply an effect of reduced a_w.

In addition, it should be noted that the Arizona appear to be heterogeneous with regard to heat resistance. The arizonae have heat resistance similar to S. typhimurium Tm-1 in unsalted media and 4.0% TSB-YE but are heterogeneous in heat resistance in TSB-YE and liquid whole egg with 10.0% NaCl. Additional evidence is provided by the similarity of thermal death kinetics, sensitivity to the NaCl "toxic effect," and overall heat resistance of S. typhimurium Tm-1 and Arizona 7 : 1,7,8 and the divergent thermal death pattern and significantly greater NaCl induced heat resistance of Arizona neotype. The Arizona neotype is not sensitive to the "toxic" heat resistance effect of NaCl. To the author's knowledge, this study is the first determination of Arizona heat resistance. It is not possible to draw broad conclusions regarding relative heat resistance of the Salmonella and Arizona groups when only a few strains have been examined. The data do point out that heat treatment that may be sufficient to destroy the Salmonella heat resistance reference strain, S. typhimurium Tm-1, may not inactivate all Arizona serotypes, specifically the Arizona neotype strain. The implications of these observations to the food industry are obvious.

The practical application of the above observations is illustrated by the characterization of NaCl induced heat resistance of the test strains in liquid whole egg containing 10.0% NaCl at 63.5°C (Figure 6, Table 5), the temperature required to pasteurize this product (1). Although the experimental technique (sealed ampule) used in this detailed study does not simulate commercial egg pasteurizing processes, it does allow precise temperature and time control. In contrast, under commercial conditions the minimal holding time may be 60 to 80% of the average holding time of 3.5 minutes (23). The complex, multi-order thermal death kinetics induced by 10.0% NaCl and subsequent extended survival of a small number of extremely heat resistant cells of all three test strains, particularly the Arizona neotype strain, reveal the inadequacy of the present pasteurization requirement. OSDA laboratory isolations of salmonellae and arizonae from salted, liquid whole eggs that have been pasteurized and repasteurized are additional evidence supporting this conclusion. Subsequent studies of Lineweaver, Garibaldi and Ijichi, (33) indicate that S. typhimurium Tm-1 and Arizona 7 : 1,7,8 (3840) have similar heat resistance and appear to be as resistant in salted whole egg as salted egg yolk.

The methods of calculating D values and extrapolating survival curves commonly used in heat resistance studies and thermal process calculations (3, 7, 21, 23, 24, 25, 38) are further challenged by the results of this study, as they have been by other investigators (7, 36, 37, 38). The failure to determine the small, extremely heat resistant populations of salmonellae and arizonae which might survive a thermal process, could lead to incorrect estimation of their survival characteristics and incorrect thermal processing requirements for various food products, particularly foods containing NaCl or having low a_w, with serious public health consequences. The results of this study challenge the adequacy of the present pasteurization requirement for liquid whole egg containing 10.0% NaCl. Furthermore, the characterization of Arizona neotype heat resistance in this study should be considered in future investigations of bacterial thermal death.

SUMMARY

Several Salmonella and Arizona strains were isolated from liquid whole eggs containing 10% NaCl. These eggs had either been pasteurized, repasteurized, or had received no heat treatment. Four of the OSDA laboratory isolated strains were screened for heat resistance in TSB-YE with 0.5, 4.0, and 10.0% NaCl. The survival characteristics of one laboratory isolated Arizona strain and two reference strains: Arizona 7 : 1, 7, 8 (OSDA 3840), S. typhimurium Tm-1, and the Arizona neotype strain (ATCC 13314), were subjected to more detailed analysis in TSB-YE and liquid whole egg with and without added NaCl.

Preliminary screening revealed that the Salmonella and Arizona laboratory isolated strains have similar heat resistance in TSB-YE with 0.5 and 10.0% NaCl but greater Arizona heat resistance in TSB-YE with 4.0% NaCl. Construction of survival curves demonstrated similar heat resistance of the laboratory isolated strain and reference strains in TSB-YE with 0.5 and 4.0% NaCl but greater Arizona neotype heat resistance in 10.0% NaCl. It was concluded that the preliminary screening method was only approximate.

Characterization of NaCl induced heat resistance of Salmonella and Arizona strains in TSB-YE indicated that NaCl induces nonlogarithmic, multiordered thermal death kinetics. The greater heat

resistance of the Arizona neotype strain in 10.0% NaCl was related to the failure of 10.0% NaCl to induce a "heat sensitive" population as it did in the less heat resistant strains. A direct correlation between heat resistance and NaCl concentration or estimated a_w was not observed. The arizonae were heterogeneous in the development of NaCl induced heat resistance.

Characterization of NaCl induced heat resistance in liquid whole egg showed that the Salmonella and Arizona test strains had similar heat resistance without added NaCl. The specific effect of 10.0% NaCl in liquid whole egg was to induce distinct "heat sensitive" and "heat resistant" populations within the test strain suspensions. The Arizona neotype strain had roughly three times the survival time of the other test strains in liquid whole egg with 10.0% NaCl at 57°C.

Ten percent NaCl also induced multiorder thermal death kinetics in liquid whole egg at 63.5°C, the required pasteurization temperature for this egg product. The extended survival of a small number of heat resistant cells of all three test strains, particularly the Arizona neotype strain, revealed the inadequacy of the present pasteurization time and temperature for liquid whole egg with 10.0% NaCl. The common methods of calculating D values and extrapolating survival curves used in heat resistance studies and thermal processing of foods are challenged. It is suggested that the failure to determine the small, extremely heat resistant population of salmonellae or arizonae that might survive thermal processing could lead to inaccurate estimation of thermal processing requirements, particularly for foods containing NaCl or low a_w, with serious public health consequences.

To the author's knowledge, this is the first report of Arizona heat resistance data.

BIBLIOGRAPHY

1. Agricultural Research Service, U. S. Department of Agriculture. Egg Pasteurization Manual. Albany, California, February 1969. 47 p.

2. American Society for Microbiology, Subcommittee on Enterobacteriaceae. Report in American Society for Microbiology News 30:22. 1964.

3. Baird-Parker, A. C., Margery Boothroyd and E. Jones. The effect of water activity on the heat resistance of heat sensitive and heat resistant strains of Salmonellae. Journal of Applied Bacteriology 33:515-522. 1970.

4. Carpenter, K. International committee on nomenclature of bacteria, subcommittee on the taxonomy of the Enterobacteriaceae. Minutes of meeting, 9 August, 1970. International Journal of Systematic Bacteriology 21:129. 1971.

5. ______________. Report and minutes of the meeting of the international Enterobacteriaceae subcommittee, Moscow, 1966. International Journal of Systematic Bacteriology 18:191-196. 1968.

6. Cotterill, O. J. and J. Glauert. Pasteurization requirements to destroy Salmonella in egg products containing sugar and salt. Poultry Science 46:1248. 1967.

7. ______________. Thermal resistance of Salmonellae in egg yolk products containing sugar or salt. Poultry Science 48:1156-1166. 1969.

8. Dabbah, Roger, W. A. Moats and V. M. Edwards. Heat survivor curves of food-borne bacteria suspended in commercially sterilized whole milk. I. Salmonellae. Journal of Dairy Science 54: 1583-1587. 1971.

9. Dauer, C. C. Summary of Disease Outbreaks and a 10-year resume. U. S. Public Health Service Report 76:915. 1960.

10. Edwards, P. R., Mary A. Fife and Carolyn H. Ramsey. Studies on the Arizona group of Enterobacteriaceae. Bacteriological Reviews 23:155-174. 1959.

11. Edwards, P. R. and W. H. Ewing. Identification of Enterobacteriaceae. Rev. ed. Minneapolis, Burgess, 1962. 258 p.

12. Elliker, P. R. and W. C. Frazier. Influence of time and temperature of incubation on heat resistance of Escherichia coli. Journal of Bacteriology 36:83-98. 1938.

13. Ewing, W. H. An outline of nomenclature for the family Enterobacteriaceae. International Bulletin of Bacteriological Nomenclature and Taxonomy 13:95-110. 1963.

14. ______________. Arizona Hinshawii Comb. Nov. International Journal of Systematic Bacteriology 19:1. 1969.

15. ______________. Enterobacteriaceae: taxonomy and nomenclature. Atlanta, Georgia, 1966. (U. S. Department of Health, Education and Welfare, National Communicable Disease Center).

16. ______________. Revised definitions for the family Enterobacteriaceae, its tribes and genera. Atlanta, Georgia, 1967. 12 p. (U. S. Department of Health, Education and Welfare, National Communicable Disease Center).

17. Ewing, W. H. and M. M. Ball. The biochemical reactions of members of the genus Salmonella. Atlanta, Georgia, 1966. 82 p. (U. S. Department of Health, Education and Welfare, National Communicable Disease Center).

18. Ewing, W. H. and M. A. Fife. A summary of the biochemical reactions of Arizona arizonae. International Journal of Systematic Bacteriology 16:427-433. 1966.

19. Ewing, W. H., M. A. Fife and B. R. Davis. The biochemical reactions of Arizona arizonae. Atlanta, Georgia, 1965. (U. S. Department of Health, Education and Welfare, National Communicable Disease Center).

20. Foster, E. M. The control of Salmonellae in processed foods: a classification system and sampling plan. Journal of the Association of Official Analytical Chemists 54:259. 1971.

21. Garibaldi, J. A. Acetic acid as a means of lowering the heat resistance of Salmonella in egg yolk products. Food Technology 22:1031-1033. 1968.

22. Garibaldi, J. A., H. Lineweaver and K. Ijichi. Number of Salmonellae in commercially broken eggs before pasteurization. Poultry Science 48:1096-1101. 1969.

23. Garibaldi, J. A., R. P. Straka and K. Ijichi. Heat resistance of Salmonella in various egg products. Applied Microbiology 17: 491. 1969.

24. Goepfert, J. M. The interrelationship between pH, temperature, oxygen supply, and available water, on growth and survival of Salmonella organisms, with special reference to confectionary products. Progress report to the National Confectioners Educational and Scientific Foundation, 1970.

25. Goepfert, J. M., I. K. Iskander and C. H. Amundson. Relation of the heat resistance of Salmonellae to the water activity of the environment. Applied Microbiology 19:429-433. 1970.

26. Hansen, N. H., and H. Reimann. Factors affecting the heat resistance of nonsporing organisms. Journal of Applied Bacteriology 26 (3):314-333. 1963.

27. Hermann, G. J. Assistant Chief, Enterobacteriology Unit, Center for Disease Control. Personal communication, Atlanta, Georgia. April 2, 1971.

28. International Association of Microbiological Societies, Enterobacteriaceae Subcommittee. International Bulletin of Bacteriological Nomenclature and Taxonomy 18:191. 1968.

29. Kauffmann, F. On the classification and nomenclature of the family Enterobacteriaceae. International Bulletin of Bacteriological Nomenclature and Taxonomy 13:187-193. 1963.

30. ________________. On the species definition. International Bulletin of Bacteriological Nomenclature and Taxonomy 13:181-186. 1963.

31. ________________. The bacteriology of Enterobacteriaceae. 3d ed. Baltimore, Williams and Wilkins, 1966. 400 p.

32. ________________. Zur differentialdiagnose der Salmonella-subgenera I, II und III. Acta Pathologia et Microbiologia Scandinavia 58:109-113. 1963.

33. Lineweaver, H. Chief, Poultry Laboratory, Western Regional Research Center, U. S. Department of Agriculture. Personal communication, Albany, California. February 1, 1972.

34. Martin, W. J. et al. Biochemical reactions of Salmonella with emphasis on the differentiation of this genus and the genera Arizona and Citrobacter. The Public Health Laboratory 27: 62-78.

35. Martin, W. J., M. A. Fife and W. H. Ewing. The occurrence and distribution of the serotypes of Arizona. Atlanta, Georgia, 1967. 23 p. (U. S. Department of Health, Education and Welfare, National Communicable Disease Center).

36. Moats, W. A. Kinetics of thermal death of bacteria. Journal of Bacteriology 105:165-171. 1971.

37. Moats, W. A., Roger Dabbah and V. M. Edwards. Interpretation of nonlogarithmic survivor curves of heated bacteria. Journal of Food Science 36:523-526. 1971.

38. ________________. Survival of Salmonella anatum heated in various media. Applied Microbiology 21:476-481. 1971.

39. Murphy, W. J. and J. F. Morris. Two outbreaks of gastro-entenitis apparently caused by a paracolon of the Arizona group. Journal of Infectious Diseases 86:255-259. 1950.

40. National Research Council, Committee on Salmonella. An evaluation of the Salmonella problem. Washington, D. C., National Academy of Sciences, 1969. 207 p.

41. Ng, Henry, Henry G. Bayne and John A. Garibaldi. Heat resistance of Salmonella: the uniqueness of Salmonella senftenberg 775 W. Applied Microbiology 17:78-82, 1969.

42. Osborne, W. W., R. P. Straka and H. Lineweaver. Heat resistance of strains of Salmonella in liquid whole egg, egg yolk and egg white. Food Research 19:451. 1954.

43. Stroup, John R. Malonate dulcitol lysine iron agar - a new differential medium for the identification of Salmonella, subgenera I-III. Journal of the Association of Official Analytical Chemists 55:214-218. 1972.

44. Stumbo, C. R. Thermobacteriology in Food Processing. New York, Academic Press, 1965.

45. Sugihara, T. F., K. Ijichi and L. Kline. Heat pasteurization of liquid whole egg. Food Technology 20:1076. 1966.

46. U. S. Department of Agriculture. Agricultural Statistics, 1967. Washington, D. C., U. S. Government Printing Office, 1968. 645 p.

47. U. S. Department of Agriculture Consumer and Marketing Services, Technicians Services Division. Microbiology Laboratory Handbook. Washington, D. C., U. S. Department of Agriculture, 1969.

48. Vas, K. and G. Proszt. Observations on the heat destruction of spores of Bacillus cereus. Journal of Applied Bacteriology 20:431. 1957.