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THE EFFECTS OF VARIOUS MENSTRA ON THE THERMAL RESISTANCE AND LEAKAGE OF METABOLIC PRODUCTS FROM *ESCHERICHIA COLI*

A Thesis Presented

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THE EFFECTS OF VARIOUS MENSTRA
ON THE THERMAL RESISTANCE AND LEAKAGE
OF METABOLIC PRODUCTS FROM ESCHERICHIA COLI

(September 1973)

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The effect of various menstra on the thermal resistance and leakage of metabolic products from Escherichia coli was investigated. The menstra tested were: 0.01 M phosphate buffer pH 7.0, 15.4% glycerol pH 7.0, 39.6% glycerol pH 7.0, 0.01 M citric acid pH 7.0, 0.01 M magnesium acetate pH 7.0, 0.85% sodium chloride pH 7.0, 8.5% sodium chloride pH 7.0, 0.85% sodium chloride pH 5.4 and 8.5% sodium chloride pH 5.4. The thermal resistance studies demonstrated a great amount of variation in the resistance of Escherichia coli among the menstra tested. In general high concentrations of glycerol, or sodium chloride and slightly acid pH were found to increase thermal resistance. The mechanism of thermal damage to cells was also investigated at 50 C and 60 C. There was leakage of 260 mu absorbing material from cell suspensions at both temperatures. However, the pattern of response varied at the two temperatures and among the menstra studied, with total leakage being less at 60 C than at 50 C although there is greater membrane damage at the higher temperature. Glycerol and sodium chloride were observed to prevent

leakage at 50 C in comparison to the other menstra but not at 60 C. The results are discussed in relation to the biochemical effects of moist heat on the organism.

INTRODUCTION

Heat destruction of microorganisms involves the destruction of life at the single cell level. When populations of microorganisms in suspension have been exposed to a stress losses, in viable number occur. The exact reasons for this are still debated, but it can be predicted that the future study of the death of single cells will mandate precise information of the effect of heat on vital cell molecules, i.e., what sequence of events leads from exposure to lethal heat energy and culminates in the cessation of organized cellular metabolism.

For the most part past studies at the single cell level have dealt primarily with the effect of extracellular factors (pH, water availability, age of cultures, etc.). It is much more important however to understand the basic mechanism that produces death in correlation with an extracellular factor so that destruction rates can be predicted.

The mechanisms proposed for heat destruction of bacteria include coagulation of protein, inactivation of enzymes, disruption of cellular lipids and damage to the genetic apparatus. While this paper is not intended to support any of these, it is an attempt to present thermal death rate data of Escherichia coli in various menstra and to examine the leakage of certain products.

LITERATURE REVIEW

The thermal resistance of bacteria is influenced by a number of factors, i.e., the composition of the culture medium in which the organisms are grown before heating, the composition of the recovery medium, the menstrum in which the organisms are heated, the density of the suspension, and the time and temperature of incubation before and after heating. Variables such as pH, the presence of certain anions and cations, the age of the culture, and various experimental procedures have also been shown to have an influence on thermal resistance. Because of this complexity, it is extremely difficult to define the factors affecting thermal resistance from a survey of the literature. A detailed analytical review of the published reports in this area is nevertheless essential for the reader to evaluate the conclusions presented in this study.

Ling[46] has shown that water in cells exists as polarized multilayers. This water state will be in equilibrium with that in the external environment. The amount of water present external to the cell is of crucial importance, both to the amount in the cell and its physical state.

It is known that the heat resistance of microbial cells increases with decreasing humidity. Precht and Hensel[56] showed that superheated steam acts as dry air, and at 140 F-150 F has less killing effect than wet steam at 100 F. It is thought that heat destruction of

microbial cells is due to inactivation of nucleic acids, enzymes, or other essential proteins present in the cells. Proteins are more stable in the dry state, thus, the effect of water on heat resistance can be explained by protein stability. If the assumption is made that water in close contact with the protein molecules determines heat resistance, then chemically bound water present as a monomolecular layer on the peptide chains is probably less important than water of hydration. This hydration water is attached to groups within, or at the surface of, protein molecules having free charges and also, though less strongly, to dipoles such as CO and NH_2 groups.

Precht and Hensel[56] have also demonstrated that free SH groups are formed when wet proteins are heated and the water binding capacity increases. Breakage of S-S bonds and hydrogen bonds is caused by thermic vibration; the peptide chains which now become mobile establish new bonds but only if water is present. With the decrease in water content the number of water dipoles between polar groups of peptide chains becomes smaller. It has been suggested that the resulting interaction of protein dipoles has a stabilizing effect so that more energy is required to unfold the peptide chains. This gives rise to increased heat resistance.

Yesair, Bohrer and Cameron[80] reported that small changes in water content may have a considerable influence on survival. This was supported by experimental data in which the heat resistance of dried bacteria was found to be much higher than those heated under moist conditions.

Murrell and Scott,[52] Calhoun and Frazier,[17] Goepfert, Iskander and Amundson,[30] Baumgartner and Wallace,[10] Schmidt,[67] and Baumgartner and Hersom[9] demonstrated that bacteria heated in a solution in which the water activity (aw) has been decreased by the addition of soluble carbohydrates exhibited an increase in heat resistance. Water activity, as defined by Frazier,[28] is the vapor pressure of the solution divided by the vapor pressure of the solvent. Water activity can be adjusted by the use of solutes and ions that reduce the number of available water molecules in solution. Thus, carbohydrates and salts have the effect of producing a drying state. Conflicting evidence, offered by Scott,[68] indicates that the reasons for the relationship between certain organisms and solutes is unknown. It has also been shown by Beilingson,[11] Rahn,[61] and Fay[27] that proteins are not coagulated by heat in the presence of carbohydrates. However, this factor was determined to be dependent upon the concentration of the carbohydrate and the amount of previous exposure.

The relationship between heat resistance and phase of growth has been long known. Cells in the log phase generally are less resistant. Sherman and Albus[69] coined the term "physiological youth" to describe these young cells. The phenomena of decreased resistance during exponential growth has been demonstrated with various bacteria by Sherman and Cameron,[70] Robertson,[63] Stark and Stark,[72] Watkins and Winslow,[82] and Strange and Shon.[74] Elliker and Frazier[24] studied the influence of time and incubation on the resistance of *E. coli* and noted that thermoresistance decreased from the time of inoculation. As the rate of reproduction diminished at the end of logarithmic growth there was a corresponding rise in thermal resistance until a peak was attained in the stationary

phase. These facts were substantiated by Anderson and Meanwell[6] and Lamana[41] who demonstrated an increase in resistance following prolonged incubation time.

Acid or alkaline reactions have been incriminated as increasing heat denaturation of protein and decreasing the heat resistance of bacteria. The heat resistance generally has an optimum within a rather narrow pH range (6.0-8.0) outside which the resistance falls rapidly as described by Bigelow and Esty,[13] Esty and Meyer,[26] and Anellis, Lubas and Raymon.[7]

White[84] found that the maximum survival of Streptococcus faecalis strains usually occured at pH 6.8. Jordan and Jacobs[36] reported a similar phenomenon with E. coli. Krishna et al.[39] stated that lactic streptococci showed a minimum heat resistance at pH 5.0 and maximum heat resistance at pH 8.0.

These results suggest that the heat resistance of bacteria generally is at a maximum at a pH very close to neutral. Levine and Fellers,[43] Schelhorn,[65] Schmidt,[67] and Baumgartner and Hersom[9] have found that varying the pH within a range of 4.5 to 6.8 has very little effect on heat resistance.

The difficulty in assessing the effect of pH on heat resistance may be due to the pH in a microbial cell differing from that of the surrounding medium. At pH values close to neutrality most of the ampholytes in the cell will have negative charges and attract hydrogen ions, thus changing the pH in the vicinity of the cell proteins. The presence of an electrolyte, such as sodium chloride, will also have an influence on pH because sodium ions tend to displace hydrogen ions from

the vicinity of the negatively charged ampholytes. Therefore, the presence of salt and other electrolytes must be taken into account when evaluating the effect of pH on heat resistance.

It has been stated by Jenson[37] that salt exerts a protective action against heat. Baumgartner and Hersom[9] however demonstrated the variability of this effect and its dependence upon the type of salt, the concentration, the suspending medium and test organism. Salts may influence the microbial cell and its heat resistance in several ways, however it is virtually impossible to predict the effect. Mitchell[48] concluded that salts may affect the pH regulation of the cell by changing the transport of acid through the osmotic barrier. Likewise, they may establish a more favorable osmotic pressure difference between the cell interior and the suspending menstrum causing decreased leakage of essential components from the cells while heating. Some 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. Precht and Hensel[56] and Strange and Shon[74] found that divalent cations such as Ca^{++} and Mg^{++} may increase the heat resistance by linking the proteins together to form heat resistant complexes. Also, high concentrations of soluble salts decrease the water activity and increase the resistance of bacterial cells by a mechanism which corresponds to the effect of drying.

Since there is no convenient way to count dead cells, the measurement of heat resistance is indirectly being predicated on survival curves. When the numbers of specimans surviving exposure to various heating periods are plotted on semilogarithmic paper, the resulting curve is usually a straight line demonstrating the progressive death of

the population. Wyss[85] determined that the shape of the survival curve suggests death of the cells is caused by a monomolecular reaction, but there is no reason to believe monomolecular reactions are concerned in bacterial death any more than any other theory. The experiments of Kronig and Paul[40] which preceded Chick[20] in 1910 demonstrated that the death of microbial cells was a logarithmic function. The logarithmic survivor curve indicates that a constant fraction of cells is destroyed per unit time. The basis of this type of curve stems from the assumption that the chance of an organism being killed is independent of its previous history of exposure to the killing agent. Two factors could lead to such a result. The first is that death results from a chance contact of the lethal agent (heat) with some sensitive site in the cell so minute that the contact occurs only on rare occasions. Such a site may be a gene or any other component that may result in a lethal mutation. This implies that the impact of heat should not be thought of as a continuous process, but as the effect of individual quanta of energy which are not homogeneously distributed in space.

A second explanation is that the resistance of each cell is not constant, but rises in a rhythmic pattern. At any moment, a definite percentage of the cells having resistance below that necessary to ensure survival are killed by the impact.

Bigelow,[12] using data gathered by Bigelow and Esty[13] while investigating the thermal death point of typical thermophilic organisms, supported this by demonstrating that (a) the hydrogen-ion concentration influences the time necessary to destroy a known suspension of spores at a given temperature, (b) the initial concentration of spores

affects the time necessary to sterilize a medium of known hydrogen-ion concentration, and (c) the larger the number of spores present in a medium the longer the time necessary to destroy them.

The straight semilog survivor curve was described by Katzin, Sandholzer and Strong[38] as the "D" value, or decimal reduction time; the time required to kill 90% of the cells present or transverse one log cycle. When logarithms of the D value are plotted against the heating temperatures a straight line is usually obtained which has been termed by Bigelow[12] as the thermal death curve. The slope of this line, the z value, represents the increase in temperature required to reduce the D value to one tenth of its value at the lower temperature.

Precht and Hensel[56] demonstrated that the z value cannot be constant over a wide temperature range because at some temperature the D value must be infinite and the z value zero. They also demonstrated that the z value is not as dependent as the D value on the composition of the heating monstrum and other variables connected with heating.

This is substantiated in the work done by Watkins and Winslow,[82] Hinshelwood[33] and Precht and Hensel[56] who determined that the different slope survivor curves are the result of a non-uniform distribution of heat resistance. On this basis the cause of death can be considered as accumulative damage to cells: the most resistant one will survive longest.

Since the thermal curves can be considered as first order biomolecular reactions which are dependent upon one variable, a mathematical equation was devised to account for both of the reactants depending on their concentration over a period of time. This equation, devised by Stumbo,[77] is

$$k = \frac{2.303}{t} \log \frac{C_o}{C}$$

in which C_o equals the concentration of reactants and C equals the concentration after reaction time t. From this equation and the research of Katzin the time required to destroy 90% of the cells is the time to transverse one log cycle. The slope of the survivor curve was determined to be

$$D = \frac{t}{\log N^o - \log N} .$$

The methods used by Stumbo[77] to determine N were either the Most Probable Number or by direct count. This was modified somewhat later by the Stumbo, Murphy, Cochran[78] method which incorporated the Halvorson and Ziegler equation for calculating the Most Probable Number. Following this, the D value for each t and N can be calculated. The use of this equation was found to increase the accuracy of the number of survivors. An opponent of this method was Schmidt[67] whose method for analyzing end point data employs an analytical plus graphical procedure. Both of these methods have been found by Reynolds and Lichtenstein[62] to be in close agreement.

The microbiologist has been actively measuring the heat destruction rates of microorganisms for about fifty years. However, there are two methods in use today for studying the resistance of bacteria to temperatures below the boiling point of water. The first is the multiple replicate unit testing cyster which Bigelow and Esty[13] first described in which suspensions of microorganisms were heated sealed in Pyrex glass

tubes. While Stumbo[76] has stated that this method is simple and employs inexpensive equipment, the fact remains that heating and cooling lags in these tubes are appreciable, and the accuracy of this type of system can only be expected when an appreciable amount (more than 5 to 10%) of the total lethal effect is due to temperatures very much more than one degree below the temperature of the heating bath. The second method, devised by Levine, Buchanan and Lease,[44] employs the use of a Woulff bottle with three necks. For use in thermal destruction studies a small mechanical stirrer is introduced through one neck, a thermometer through a second, while the third serves for the introduction and withdrawal of the samples. Stumbo[77] has stated that the chief advantages of this method are:

1. "It is a convenient method for determining the resistance of 'low resistance' organisms.
2. It requires only readily available equipment.
3. Heating and cooling lags are negligible when the method is properly employed."

The disadvantages of this method are negligible, and, when compared to the multiple replicate testing system, proves to be more reliable.

The exact mechanism whereby nonsporing bacteria are affected by thermal damage is unknown, however, there are several changes observed in heated bacterial suspensions and any one of these, or combinations thereof, could theoretically be the cause for thermally induced death. Mechanisms proposed for heat destruction of bacteria include coagulation of protein, inactivation of enzymes, disruption of cellular lipids, damage to the genetic apparatus, leakage of intracellular constituents and breakdown of ribonucleic acid.

Chick[20] proposed protein denaturation as the cause of death during heating. This theory was further supported by Rahn,[58,59,60] who believed that "the loss of reproductive power of a bacterial cell when subjected to moist heat is due to the denaturation of one gene essential to reproduction." Rahn[58,59,60] believed that since the death of microorganisms follows a unimolecular or first order biomolecular reaction, death of a single cell was subsequently the result of denaturation of a single molecule in degrees. Fricke and Demerer[29] substantiated this theory when they demonstrated that the size of a gene is that of a small protein molecule, and thus a gene could consist of only one or two molecules. However, Rahn and Schroeder[59] stated that enzyme coagulation was not the cause of death by demonstrating that where 99% of a bacterial population was destroyed by heat, only 14% of the peroxidase and 10% of the catalase was inactivated. This was further substantiated by Califano[18] who found that the lethal effect on microorganisms is due to a separation of ribonucleic acid. Thus, as stated by Ogensky[53] "... We do not know whether the logarithmic order of death is an expression of the monomolecular reaction of protein denaturation or of a subsequent phenomenom." Ogensky's theory was supported by Ingraham[35] and Rahn[60] who demonstrated that the enzyme content of the cells remains constant and Ingraham,[35] who suggested that the order of thermal death was dependent upon the lethal effect of an essential gene or a structure, such as a cell membrane.

There has been a great deal of biochemical data accumulated to indicate that the cytoplasmic membrane is damaged during heating. Horita and Burton[50] demonstrated alterations in the activity of malic

dehydrogenase was due to heat induced permeability changes in Vibrio marinus. Haight and Morita[31] found leakage of various cellular components (DNA, RNA, and amino acids) associated to the viability of Vibrio marinus HP-1 when exposed to temperatures above its maximal temperature for growth (20 C). Likewise, Russell and Harries[64] showed that leakage of 260 mu absorbing material preceded loss of viability of E. coli when thermally treated, indicative of membrane damage. Allwood and Russell[2,3,4,5] also demonstrated the leakage of 260 mu absorbing material and found a direct relationship between death and leakage both of which increased with increased temperature up to 50 C. They later demonstrated that although no leakage of DNA occurred there is evidence to indicate that RNA degradation is linked with thermally induced death in nonsporing bacteria. Allwood and Russell[4] also demonstrated cytoplasmic damage by the penetration of 8-anilino-1-napthaline-sulphonic acid and into heated cells.

Califano,[18] Eignor,[23] Strange and Shon,[74] and Prostgate and Hunter[57] demonstrated the involvement of RNA degradation when microorganisms are heated. The work of Iandolo and Ordal[34] and Sorgin and Ordal[71] demonstrated that the ability of heat damaged Staph. aureus to grow on agar containing 7½ sodium chloride was due to regeneration of ribosomes and not protein synthesis.

Thus, it appears that RNA is involved both in the mechanism of thermophily and the early events of heat damage. This was indicated by Sorgin and Ordal[71] who found nucleic acid to be chiefly involved in thermal death. They separated and characterized ribosomal RNA on a methylated albumin kiesolguhr column. They further substantiated these

results by using sucrose gradient analysis and labeled uracil and glutamic acid isotopes. Clark, Wittor and Ordal[21] and Pace and Campbell[54] related ribosomal heat stability with maximum growth temperature, and that the lag period following thermal stress involved recovery and not growth and that this recovery involved RNA synthesis.

METHODS AND MATERIALS

Bacterial Strain

Escherichia coli (A.T.C.C. #11775) was used throughout this study.

Culture Maintenance

In order to keep the culture uniform and free of mutants it was initially grown on Difco Trypticase Soy Broth (T.S.B.) for forty eight hours at 37 C.

It was then seeded onto individual Difco Trypticase Soy Agar (T.S.A.) slants and incubated at 37 C for 48 hours, tightly sealed and frozen. For use in the determination of the thermal death curves one slant was thawed at room temperature each day and a loop of culture was transferred into 10 ml of T.S.B. which was then incubated for 24 hours on a platform shaker. One ml of this culture was then transferred into 10 ml of T.S.B. and incubated for 24 hours at 37 C after which they were stored at 4 C until used that day.

The cell suspension for the determination of leakage products were grown in the same manner as that for the thermal death curves with one exception. The final seeding was into a 500 ml erlenmeyer flask containing 200 ml of T.S.B.

Preparation of Cell Suspensions

The cell suspension for use in the thermal death curve studies was prepared by vortexing the culture for 60 seconds and diluting 1:3 in

sterile distilled water.

The cells for the determination of leakage products were harvested in a different manner. The 24 hour T.S.B. cultures were centrifuged at 3,000 x g for fifteen minutes at 4 C on a Sorvall Superspeed RC-2 refrigerated centrifuge. This procedure was repeated three times. After the final centrifuging the pellet was washed with sterile distilled water and resuspended to the proper volume.

Assay of the Cells

Assays of viable organisms in suspension before and after heating were determined by plating employing several serial dilutions. Duplicate T.S.A. pour plates of each dilution were made, incubated at 37 C for 48 hours and counted, employing a Quebec colony counter. The results of these initial counts were used in the D value calculations and in the determination of leakage products.

Growth Curve

A growth curve of the test organism (Appendix Part II) was constructed over a 24 hour period to determine the growth characteristics of the test culture used. The data points for the curve were obtained by recording the optical density at 550 mu using a Bausch and Lomb 340 Spectrophotometer.

Control System

Control systems for both the thermal destruction data and cellular leakage experiments were included to determine what effect if any the

various menstra had on the organism. The determination of the effect of the menstra without heat was performed by inoculating test tubes containing 9 ml of sterile menstrum with one ml of a 10^{-6} dilution of E. coli. These tubes were vortexed and held at room temperature for twenty minutes. One ml and one tenth were then plated by pour plate in T.S.A. The control system used in the examination of leakage products employed the use of Woulff flasks containing the menstrum being tested. Analysis of the menstrum at room temperature was performed in exactly the same manner as the examination of leakage products on the heated menstrum.

Suspending Menstrums

Six of the menstra used, whose pH was 7.0, were buffered with 0.01 M potassium phosphate. The phosphate buffer was prepared by the addition of 0.01 M K_2HPO_4 and 0.01 M KH_2PO_4 in proportion to reach pH 7.0 as measured on a Beckman Zeromatic SS-3 pH meter. The pH of the other two menstra was obtained by the addition of sodium chloride in appropriate proportions.

The menstra used were:

a) 0.01 M citric acid pH 7.0

b) 0.01 M magnesium acetate pH 7.0

c) 0.01 M phosphate buffer pH 7.0

d) 0.85% sodium chloride pH 7.0

e) 8.5% sodium chloride pH 7.0

f) 39.6% glycerol pH 7.0

g) 15.4% glycerol pH 7.0

h) 0.85% sodium chloride pH 5.4

i) 8.5% sodium chloride pH 5.4.

The menstra containing glycerol (U.S.P. grade) were determined on a weight to weight basis. The buffering capacity of each testing was determined by measuring the final pH on each trial. The volume of the flasks was decided after a trial method in which it was determined that the flasks were to be filled with 209 ml of the menstrum before autoclaving for twenty minutes to reach the desired, final volume of 198 ml.

Thermal Testing Procedure

The method used in this study was the Mueller[51] modification of the Levine three necked Woulff flask system. This technique included the use of a copper lined heating tub into which six Woulff flasks, containing the various menstra were fitted. This technique has the advantages of using a delivery system in which machined aluminum plugs with fixed cannulas could be used, and allowing the removal of samples by means of a 2 ml automatic pipette. The organisms within the menstrum were kept suspended by submerged turbine driven magnetic stirrers and egg shaped stirring bars in the flask. This type of system, which is different from that used by Stumbo[76] allowed for more accurate and controlled stirring in which the water driven stirrers could be adjusted to precise increments thereby avoiding splashing.

The temperature within the testing flasks was controlled by thermocouples connected to a Bristol Dynamaster recording pyrometer. The thermocouples used were coordinated against a standardized type "T" Xactpak (by Industronics Inc.) thermocouple. The heating supply was provided by two copper heating elements which encompassed the bath itself.

These elements were controlled in turn by a Fisherbrand thermoregulator rotostat which was indirectly monitored by the standardized thermocouple. The circulating system employed the use of both a central circulating unit and a circulatory pump which removed water from the bottom and returned it to the top of the tank. There appeared to be no inadequacies in the temperature within the tank with this system. However, to avoid any fluctuations, only two stations were used both on either side of the circulating pump. In order to maintain the system in the tank as close to equilibrium as possible the top of the bath was covered with aluminum foil. Once the system had reached equilibrium the vortexed cells were introduced into the menstrum by the use of machined inoculating plugs containing fixed cannulas. After predetermined time intervals 1 ml aliquots were removed and plated as previously described.

Leakage Testing Procedure

Suspension of the Organism

The techniques used for the determination of leakage were similar to those employed by Allwood and Russell.[2,3,4,5] The cultures used for these experiments differed from those used in the thermal destruction experiments in that 2 ml of the culture were transferred into two 500 ml Erlenmeyer Flasks containing 200 ml of T.S.B., incubated on a platform shaker for 24 hours at 37 C. The suspension was then centrifuged at 3,000 x g and washed three times with sterile distilled water. The suspension was adjusted to a concentration of approximately 10^{10} viable cells/ml.

Heating Procedure

Two ml of the desired suspension of cells were delivered into the heating menstrums in the same manner as described above for the thermal destruction investigation.

Analysis of Cells

Samples (10 ml) were removed when required and were subjected to the following procedure. The samples, once removed, were immediately cooled and then centrifuged at 20,000 x g at 4 C; the supernatant fluid was carefully removed and examined for ultraviolet absorbing material, protein, ribose (RNA) and DNA as described below. The pellet was then treated with cold 10% TCA and recentrifuged. The supernatant was then carefully removed and analyzed as was the original supernatant.

Extracts were analyzed for ribose concentration by the Orcinol reaction (Albaum and Umbeit[76]) with D-ribose as a standard; for DNA by Burton's[5] modification of the diphenylamine reaction with Calf thymus DNA as standard. Protein concentration in the extracts were determined by Lowry's[47] modification of the Folin reaction using bovine serum albumin as a standard. Ultraviolet absorption (UV) spectra were obtained with a Beckman spectrophotometer, model DU, using a 1.0 cm light path. Colorimetric determinations were performed in matched tubes for optical cells. Concentrations of reacting materials were determined by extrapolation from standard curves which were plotted using concentrations that would follow the Beer Lambert Law.

Data Analysis

The mathematical statement used to determine the D values was that devised by Stumbo:[76]

$$D = \frac{t}{\log x} - \log y$$

where D is the time necessary to transverse one log cycle, t is equivalent to the time of heating, x the initial concentration of organisms and y the concentration of survivors. The data obtained was transformed into log values and fitted to linear regression analysis. Once the regression line was calculated from a set of data, the equation to predict the value of the response variable (ninety-five percent confidence intervals) for the D value at each temperature was calculated. In addition the coefficient of correlation for each line was determined to measure the intensity of the association between D values and temperature for each line. The "t" distribution test was also performed to determine the probability of a chance occurrence. Since all of the lines constructed shared a common data point at 143.7 F analysis of variance was conducted with the D values at this point. This test is an estimate of variance among the levels of the tested factor against a pooled estimate of variance within the levels of the factor. The use of this method requires three assumptions: (a) that there is only one factor which determines the changing variable, (b) that the effects of chance and of non tested factors are equally and normally distributed at all levels of the tested factor, and (c) that each testing made at each temperature level is independent and not affected in any other fashion by any carrier

over other levels.

RESULTS

The data on Table 1 indicates that the numbers of E. coli were not affected after a twenty minute period in all menstra at room temperature. Therefore, any leakage or destruction of organisms observed during heating of the bacterial suspensions was due to the heat input into the various systems.

The results of the thermal death studies are presented in Tables 2-10 which include the initial count, the final count, the time of the final count, the calculated D values, and their respective logs, the standard deviation of the log D values for each line, the x data points, the y data points, the coefficient of correlation, and the t-distribution value which demonstrates the precision of the thermal death curves.

The data incorporated in these tables and figures 1-9 again support the theory of the logarithmic order of death. These results were further substantiated by the high coefficient of correlation (-0.951--0.992). A t-distribution test was performed on all the data to determine the possibility of chance occurrences and it was found that the data is significant beyond the 0.05 level. Thus, the linear relationships obtained of time versus temperature are valid. The standard deviation, another indicator of accuracy, ranged from 0.015 to 0.004.

The thermal resistance data of E. coli at 143.7 F was analyzed and summarized in Table 11. These data indicate a great variation in the thermal resistance of E. coli when heated in the various menstra. These differences are also demonstrated in the thermal destruction curves (Figures 1-9). Menstra containing 39.6% glycerol in 0.01 M phosphate

TABLE 1. The effect of various menstra on E. coli at room temperature for twenty minutes.

	INITIAL COUNT CELLS/ml	MEAN FINAL COUNT CELLS/ml	PERCENT RECOVERY
1. 0.01M phosphate buffer pH 7.0	110	92	84
2. 15.4% glycerol in 0.01M phosphate buffer pH 7.0	60	45	75
3. 39.6% glycerol in 0.01M phosphate buffer pH 7.0	110	92	84
4. 0.01M citric acid in 0.01M phosphate buffer pH 7.0	120	111	92
5. 0.01M magnesium acetate in 0.01M phosphate buffer pH 7.0	120	102	85
6. 0.85% sodium chloride in 0.01M phosphate buffer pH 7.0	118	93	75
7. 0.85% sodium chloride in 0.01M phosphate buffer pH 5.4	120	96	80
8. 8.5% sodium chloride in 0.01M phosphate buffer pH 7.0	120	98	82
9. 8.5% sodium chloride in 0.01M phosphate buffer pH 5.4	120	101	84

TABLE 2. Thermal destruction calculations for E. coli in 0.01 M magnesium acetate pH 7.0.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG (D)
143.7	5000000.	13.	1.166	0.21	-0.677781
143.7	5000000.	60.	1.166	0.24	-0.619789
143.7	5000000.	15.	1.166	0.21	-0.677781
143.7	5000000.	81.	1.166	0.24	-0.619789
143.7	5000000.	82.	1.166	0.24	-0.619789
143.7	5000000.	50.	1.166	0.23	-0.638272
143.7	5000000.	40.	1.166	0.23	-0.638272
143.7	5000000.	87.	1.166	0.24	-0.619789
143.7	5000000.	64.	1.166	0.24	-0.619789
143.7	5000000.	47.	1.166	0.23	-0.638272
143.7	3670000.	54.	1.333	0.28	-0.552842
143.7	3670000.	64.	1.333	0.28	-0.552842
143.7	3670000.	31.	1.333	0.26	-0.585027
143.7	3670000.	16.	1.333	0.25	-0.602060
143.7	3670000.	28.	1.333	0.26	-0.585027
143.7	3670000.	73.	1.333	0.28	-0.552842
139.5	3670000.	26.	2.500	0.49	-0.309804
139.5	3670000.	27.	2.500	0.49	-0.309804
139.5	3670000.	30.	2.500	0.49	-0.309804
139.5	3670000.	17.	2.500	0.47	-0.327902
139.5	3670000.	37.	2.500	0.50	-0.301030
139.5	3670000.	81.	2.500	0.54	-0.267606
139.5	3670000.	90.	2.500	0.54	-0.267606

TABLE 2 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG (D)
139.5	3670000.	27.	2.500	0.49	-0.309804
139.5	3670000.	81.	2.500	0.54	-0.267606
139.5	3670000.	71.	2.500	0.53	-0.275724
139.5	4330000.	23.	2.500	0.47	-0.327902
139.5	4330000.	60.	2.000	0.41	-0.387216
139.5	4330000.	45.	2.000	0.40	-0.397940
139.5	4330000.	124.	2.000	0.44	-0.356547
139.5	4330000.	36.	2.000	0.39	-0.408935
139.5	4330000.	60.	2.500	0.51	-0.292430
139.5	4330000.	96.	2.000	0.43	-0.366632
139.5	4330000.	164.	2.000	0.45	-0.346787
139.5	4330000.	62.	2.500	0.52	-0.283997
135.4	5330000.	70.	7.000	1.43	0.155336
135.4	5330000.	47.	7.000	1.38	0.139879
135.4	5330000.	37.	7.000	1.36	0.133539
135.4	5330000.	30.	7.000	1.33	0.123852
135.4	5330000.	10.	7.000	1.22	0.086360
135.4	5330000.	15.	7.000	1.26	0.100371
135.4	5330000.	30.	7.000	1.33	0.123852
135.4	5330000.	50.	7.000	1.39	0.143015
135.4	5330000.	81.	7.000	1.45	0.161368
135.4	4000000.	76.	7.000	1.48	0.170262
135.4	4000000.	20.	7.000	1.32	0.120574
135.4	4000000.	28.	7.000	1.36	0.133539

TABLE 2 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG (D)
135.4	4000000.	57.	7.000	1.44	0.158362
135.4	4000000.	38.	7.000	1.39	0.143015
135.4	4000000.	55.	7.000	1.44	0.158362
135.4	4000000.	46.	7.000	1.42	0.152288
135.4	4000000.	87.	7.000	1.50	0.176091
135.4	4000000.	34.	7.000	1.38	0.139879
135.4	4000000.	40.	7.000	1.40	0.146128

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
139.3	-0.245349	12.472	-0.091

X VALUE	STD. DEV.	ADJ. STD. DEV.
143.7	0.008	0.013
139.5	0.008	0.008
135.4	0.008	0.012

DATA POINTS	X	Y	95% Confidence interval
	143.7	-0.605	-0.631....-0.579
	139.5	-0.220	-0.238....-0.206
	135.4	+0.151	+0.157....+0.145

The coefficient of correlation is -0.984.

The T-distribution value is 3.576.

Significant beyond 0.001 level of significance.

FIGURE 1. Thermal destruction curve of E. coli in 0.01 M magnesium acetate pH 7.0.

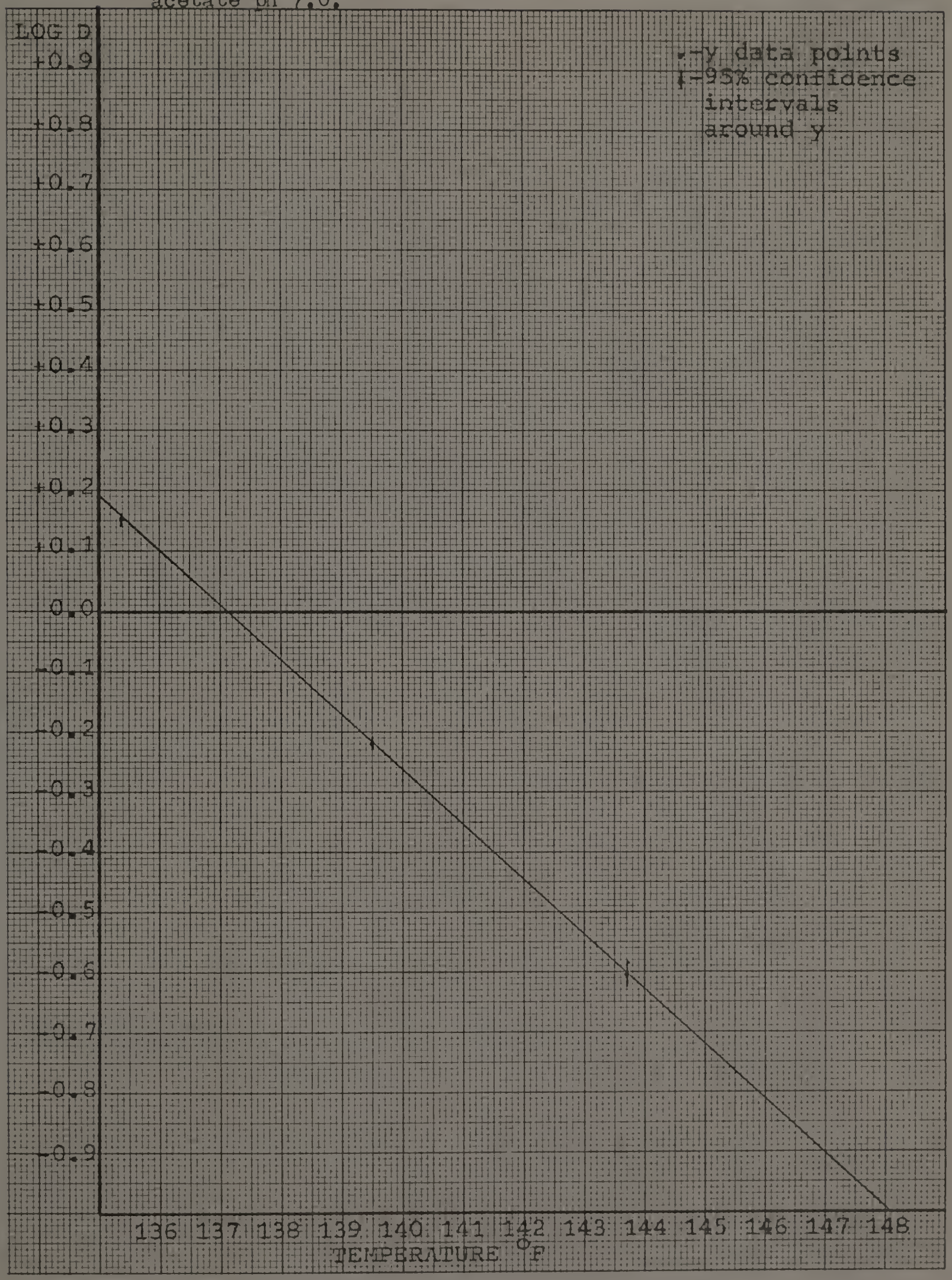


TABLE 3. Thermal destruction calculations for E. coli in 0.01 M citric acid pH 7.0.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
143.7	5000000.	84.	1.750	0.37	-0.431798
143.7	5000000.	34.	1.750	0.34	-0.468521
143.7	5000000.	65.	1.750	0.36	-0.443697
143.7	5000000.	50.	1.750	0.35	-0.455932
143.7	6330000.	25.	1.750	0.32	-0.494850
143.7	6330000.	50.	1.750	0.34	-0.468521
143.7	6330000.	52.	1.750	0.34	-0.468521
143.7	6330000.	108.	1.750	0.37	-0.431798
143.7	6330000.	57.	1.750	0.35	-0.455932
143.7	6330000.	59.	1.750	0.35	-0.455932
143.7	6330000.	63.	1.750	0.35	-0.455932
139.5	3670000.	22.	2.500	0.48	-0.318759
139.5	3670000.	23.	2.500	0.48	-0.318759
139.5	3670000.	55.	2.500	0.52	-0.283997
139.5	3670000.	23.	2.500	0.48	-0.318759
139.5	3670000.	30.	2.500	0.49	-0.309804
139.5	3670000.	46.	2.500	0.51	-0.292430
139.5	3670000.	79.	2.500	0.54	-0.267606
139.5	3670000.	57.	2.500	0.52	-0.283997
139.5	3670000.	52.	2.750	0.57	-0.244125
139.5	3670000.	56.	2.500	0.52	-0.283997
139.5	4330000.	14.	2.750	0.50	-0.301030
139.5	4330000.	14.	2.500	0.46	-0.337242

TABLE 3 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
139.5	4330000.	17.	2.500	0.46	-0.337242
139.5	4330000.	24.	2.500	0.48	-0.318759
139.5	4330000.	14.	2.500	0.46	-0.337242
139.5	4330000.	20.	2.500	0.47	-0.327902
139.5	4330000.	20.	2.500	0.47	-0.327902
139.5	4330000.	49.	2.500	0.51	-0.292430
139.5	4330000.	25.	2.500	0.48	-0.318759
139.5	4330000.	83.	2.500	0.53	-0.275724
135.4	3000000.	16.	8.000	1.52	0.181844
135.4	3000000.	12.	8.000	1.48	0.170262
135.4	3000000.	87.	8.000	1.76	0.245513
135.4	3000000.	79.	8.000	1.75	0.243038
135.4	3000000.	73.	9.000	1.95	0.290035
135.4	3000000.	28.	9.000	1.79	0.252853
135.4	3000000.	14.	8.000	1.50	0.176091
135.4	3000000.	17.	9.000	1.72	0.235528
135.4	3000000.	30.	9.000	1.80	0.255273
135.4	4670000.	71.	10.000	2.08	0.318063
135.4	4670000.	51.	10.000	2.02	0.305351
135.4	4670000.	41.	10.000	1.98	0.296665
135.4	4670000.	66.	10.000	2.06	0.313867
135.4	4670000.	47.	10.000	2.00	0.301030
135.4	4670000.	66.	10.000	2.06	0.313867
135.4	4670000.	54.	10.000	2.03	0.307496

TABLE 3 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
135.4	4670000.	87.	10.000	2.11	0.324282
135.4	4670000.	69.	10.000	2.07	0.315970
135.4	4670000.	78.	10.000	2.09	0.320146
135.4	5330000.	34.	9.000	1.73	0.238046
135.4	5330000.	31.	9.000	1.72	0.235528
135.4	5330000.	41.	9.000	1.76	0.245513
135.4	5330000.	71.	9.000	1.85	0.267172
135.4	5330000.	47.	9.000	1.78	0.250420
135.4	5330000.	34.	9.000	1.73	0.238046
135.4	5330000.	28.	8.000	1.52	0.181844
135.4	5330000.	39.	8.000	1.56	0.193125
135.4	5330000.	35.	8.000	1.54	0.187521
135.4	5330000.	12.	9.000	1.59	0.201397
135.4	3000000.	80.	9.000	1.97	0.294466
135.4	3000000.	62.	9.000	1.92	0.283301
135.4	3000000.	79.	9.000	1.97	0.294466
135.4	3000000.	51.	10.000	2.10	0.322219
135.4	3000000.	34.	9.000	1.82	0.260071
135.4	3000000.	45.	9.000	1.87	0.271842
135.4	3000000.	65.	9.000	1.93	0.285557
135.4	3000000.	97.	9.000	2.00	0.301030
135.4	3000000.	61.	9.000	1.92	0.283301
135.4	3000000.	40.	9.000	1.85	0.267172

TABLE 3 continued

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
137.9	-0.012267	13.396	-0.097

X VALUE	STD. DEV.	ADJ. STD. DEV.
143.7	0.012	0.025
139.5	0.012	0.013
135.4	0.012	0.015

DATA POINTS	X	Y	95% Confidence interval
	143.7	-0.543	-0.493....-0.592
	139.5	-0.146	-0.120....-0.172
	135.4	+0.262	+0.232....+0.292

The coefficient of correlation is -0.951.

The T-distribution value is 4.019.

Significant beyond 0.001 level of significance.

FIGURE 2. Thermal destruction curve of E. coli in 0.01 M citric acid pH 7.0.

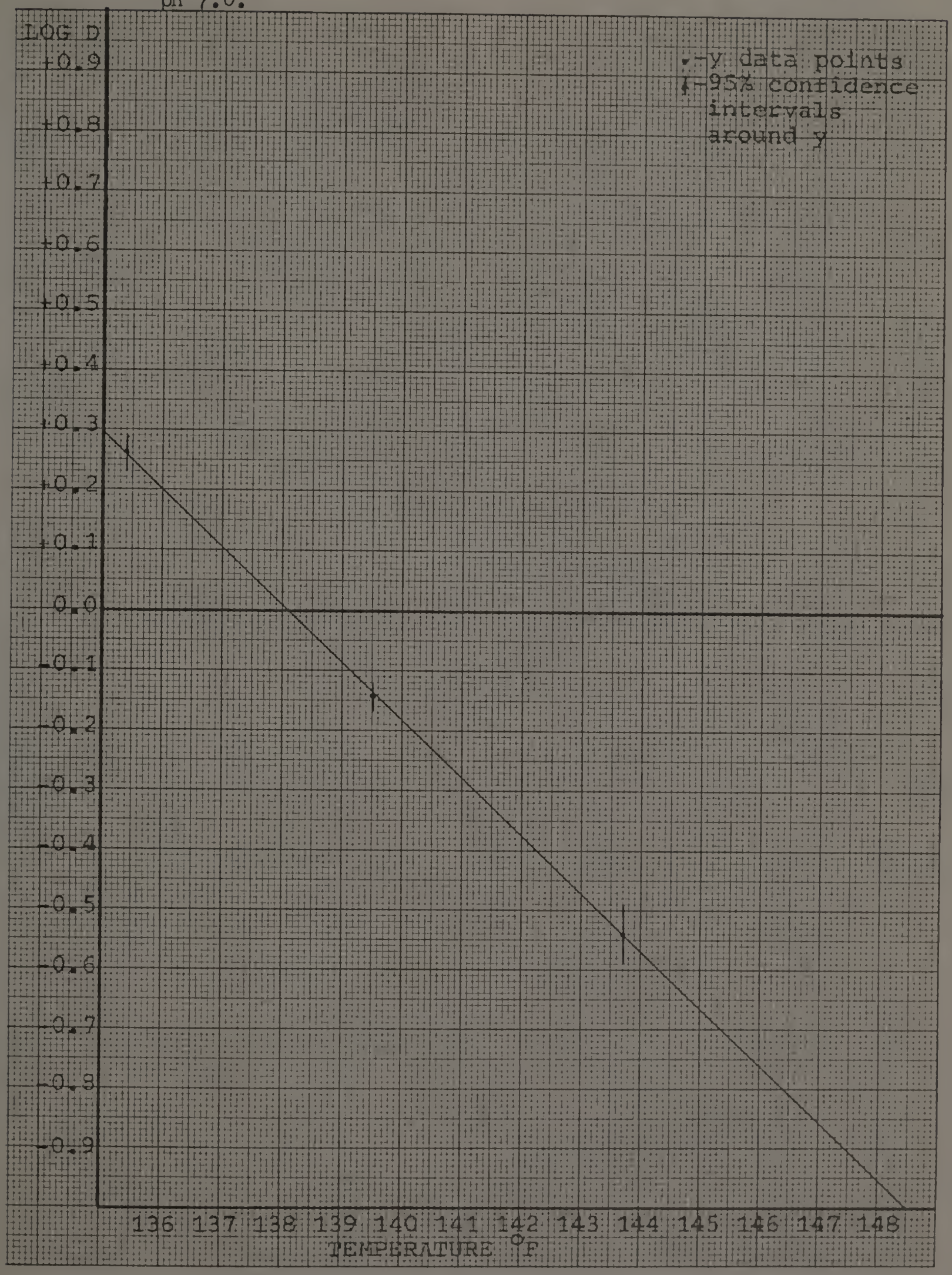


TABLE 4. Thermal destruction calculations for E. coli in 0.85% sodium chloride pH 5.4.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
143.7	6330000.	20.	1.333	0.24	-0.619789
143.7	6330000.	16.	1.333	0.24	-0.619789
143.7	6330000.	16.	1.333	0.24	-0.619789
143.7	6330000.	28.	1.500	0.28	-0.552842
143.7	6330000.	26.	1.500	0.26	-0.585027
143.7	6330000.	26.	1.500	0.28	-0.552842
143.7	6330000.	60.	1.500	0.30	-0.522879
143.7	6330000.	47.	1.333	0.26	-0.585027
143.7	6330000.	54.	1.333	0.26	-0.585027
143.7	6330000.	11.	1.333	0.23	-0.638272
143.7	5330000.	56.	1.500	0.30	-0.522879
143.7	5330000.	45.	1.500	0.30	-0.522879
143.7	5330000.	50.	1.750	0.35	-0.455932
143.7	5330000.	56.	1.750	0.35	-0.455932
143.7	5330000.	45.	1.500	0.30	-0.522879
143.7	5330000.	10.	1.333	0.23	-0.638272
143.7	5330000.	45.	1.500	0.30	-0.522879
143.7	5330000.	56.	1.333	0.27	-0.568636
143.7	5330000.	28.	1.333	0.25	-0.602060
143.7	5330000.	53.	1.333	0.27	-0.568636
141.8	4000000.	138.	2.500	0.56	-0.251812
141.8	4000000.	50.	2.500	0.51	-0.292430
141.8	4000000.	36.	2.500	0.50	-0.301030

TABLE 4 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
141.8	4000000.	14.	2.500	0.46	-0.337242
141.8	4000000.	46.	2.500	0.51	-0.292430
141.8	4000000.	102.	2.500	0.54	-0.267606
141.8	4000000.	130.	2.500	0.56	-0.251812
141.8	4000000.	16.	3.000	0.56	-0.251812
141.8	4000000.	16.	2.500	0.46	-0.337242
141.8	4000000.	23.	2.500	0.48	-0.318759
139.5	5330000.	26.	4.500	0.85	-0.070581
139.5	5330000.	85.	4.500	0.94	-0.026872
139.5	5330000.	54.	4.000	0.80	-0.096910
139.5	5330000.	70.	4.500	0.92	-0.036212
139.5	5330000.	76.	4.000	0.83	-0.080922
139.5	5330000.	105.	4.000	0.85	-0.070581
139.5	5330000.	40.	4.500	0.88	-0.055517
139.5	5330000.	65.	4.500	0.92	-0.036212
139.5	5330000.	27.	4.500	0.85	-0.070581
139.5	5330000.	25.	4.500	0.84	-0.075721
139.5	4330000.	18.	4.000	0.74	-0.130768
139.5	4330000.	46.	4.000	0.80	-0.096910
139.5	4330000.	52.	4.000	0.81	-0.091515
139.5	4330000.	16.	4.000	0.74	-0.130768
139.5	4330000.	16.	4.000	0.74	-0.130768
139.5	4330000.	22.	4.000	0.76	-0.119186
139.5	4330000.	14.	4.000	0.73	-0.136677

TABLE 4 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
139.5	4330000.	23.	4.000	0.76	-0.119186
139.5	4330000.	49.	4.000	0.81	-0.091515
139.5	4330000.	48.	4.000	0.81	-0.091515
139.5	4000000.	14.	4.000	0.73	-0.136677
139.5	4000000.	42.	3.500	0.70	-0.154902
139.5	4000000.	54.	4.000	0.82	-0.086186
139.5	4000000.	15.	4.000	0.74	-0.130768
139.5	4000000.	18.	4.000	0.75	-0.124939
139.5	4000000.	26.	4.000	0.77	-0.113509
139.5	4000000.	42.	4.000	0.80	-0.096910
139.5	4000000.	15.	4.000	0.74	-0.130768
139.5	4000000.	78.	4.000	0.85	-0.070581
139.5	4000000.	30.	4.000	0.78	-0.107905
135.4	5330000.	48.	11.000	2.18	0.338456
135.4	5330000.	52.	11.000	2.20	0.342423
135.4	5330000.	84.	11.000	2.29	0.359835
135.4	5330000.	93.	11.000	2.31	0.363612
135.4	5330000.	44.	11.000	2.16	0.334454
135.4	5330000.	31.	11.000	2.10	0.322219
135.4	5330000.	79.	12.000	2.48	0.394452
135.4	5330000.	97.	12.000	2.53	0.403121
135.4	5330000.	62.	12.000	2.43	0.385606
135.4	5330000.	44.	12.000	2.36	0.372912

TABLE 4 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
135.4	4000000.	88.	12.000	2.53	0.411620
135.4	4000000.	50.	11.000	2.24	0.350248
135.4	4000000.	19.	11.000	2.07	0.315970
135.4	4000000.	28.	11.000	2.13	0.328380
135.4	4000000.	57.	11.000	2.27	0.356026
135.4	4000000.	61.	11.000	2.28	0.357935
135.4	4000000.	14.	11.000	2.02	0.305351
135.4	4000000.	44.	11.000	2.22	0.346353
135.4	4000000.	17.	11.000	2.05	0.311754
135.4	4000000.	71.	11.000	2.32	0.365438

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
139.8	-0.125129	15.085	-0.109

X VALUE	STD. DEV.	ADJ. STD. DEV.
143.7	0.005	0.008
139.5	0.005	0.005
135.4	0.005	0.008
141.8	0.005	0.006

DATA POINTS	X	Y	95% Confidence interval
	143.7	-0.578	-0.560....-0.590
	141.8	-0.371	-0.380....-0.362

TABLE 4 continued

DATA POINTS	X	Y	95% Confidence interval
	139.5	-0.120	-0.135....-0.105
	135.4	+0.326	+0.338....+0.314

The coefficient of correlation is -0.992.

The T-distribution value is 4.398.

Significant beyond .001 level of significance.

FIGURE 3. Thermal destruction curve of E. coli in 0.85% sodium chloride pH 5.4.

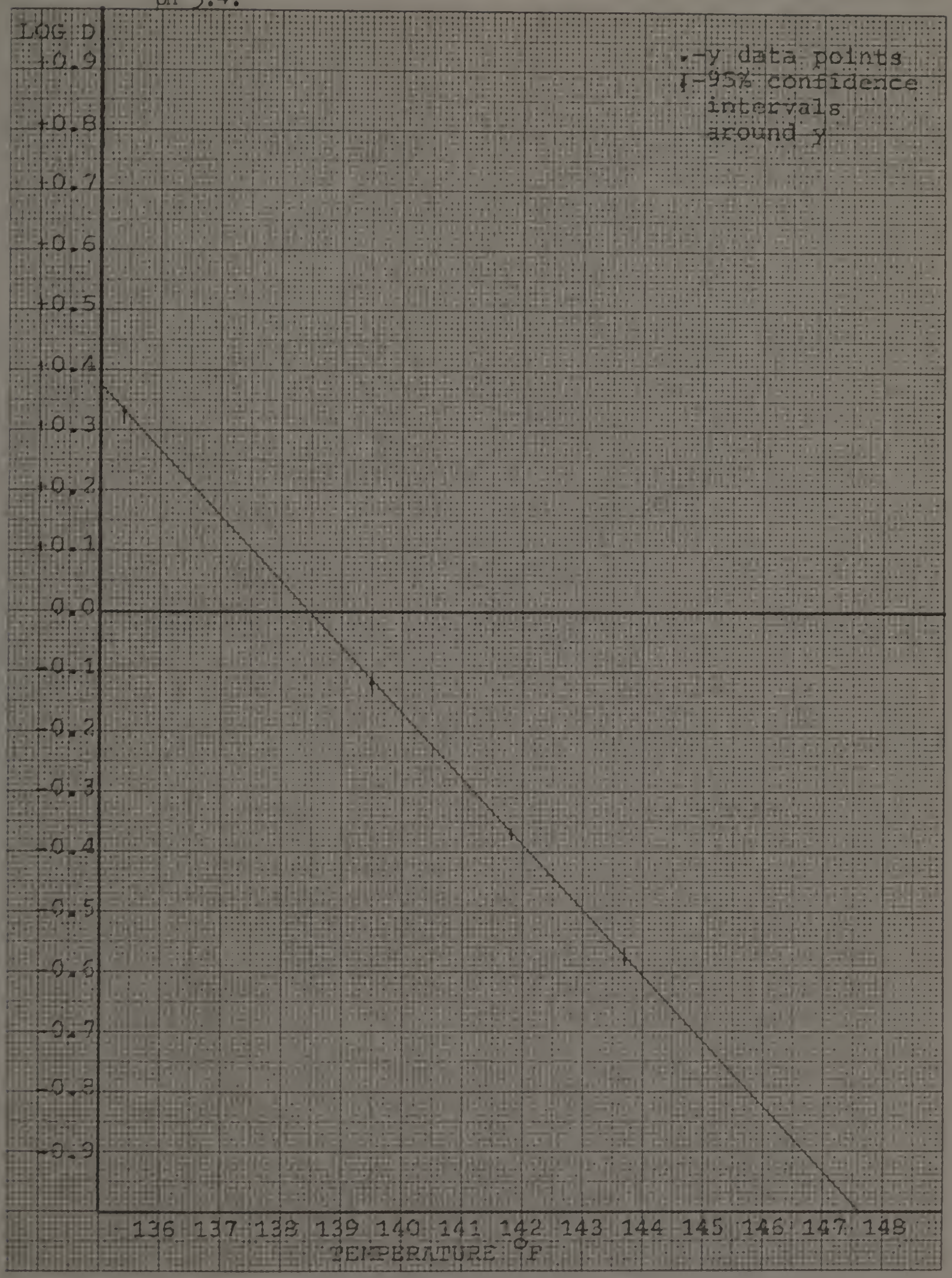


TABLE 5. Thermal destruction calculations for E. coli in 0.85% sodium chloride pH 7.0.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
141.8	4670000.	29.	1.300	0.25	-0.602060
141.8	4670000.	36.	1.300	0.25	-0.602060
141.8	4670000.	45.	1.300	0.26	-0.585027
141.8	4670000.	28.	1.300	0.25	-0.602060
141.8	4670000.	29.	1.300	0.25	-0.602060
141.8	4670000.	37.	1.300	0.25	-0.602060
141.8	4670000.	25.	1.300	0.25	-0.602060
141.8	4670000.	22.	1.300	0.24	-0.619789
141.8	4670000.	26.	1.300	0.25	-0.602060
141.8	4670000.	29.	1.300	0.25	-0.602060
141.8	4000000.	46.	1.500	0.30	-0.522879
141.8	4000000.	6.	1.500	0.26	-0.585027
141.8	4000000.	47.	1.500	0.30	-0.522879
141.8	4000000.	18.	1.500	0.28	-0.552842
141.8	4000000.	19.	1.500	0.28	-0.552842
141.8	4000000.	18.	1.500	0.28	-0.552842
139.5	5000000.	41.	2.000	0.39	-0.408935
139.5	5000000.	34.	1.500	0.29	-0.537602
139.5	5000000.	32.	2.000	0.39	-0.408935
139.5	5000000.	10.	2.000	0.35	-0.455932
139.5	5000000.	41.	2.000	0.39	-0.408935
139.5	5000000.	27.	2.000	0.38	-0.420216
139.5	5000000.	40.	2.000	0.39	-0.408935

TABLE 5 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
139.5	5000000.	55.	2.000	0.40	-0.397940
139.5	5000000.	89.	2.000	0.42	-0.376751
139.5	5000000.	47.	2.000	0.40	-0.397940
139.5	4000000.	80.	2.000	0.43	-0.366532
139.5	4000000.	26.	2.000	0.39	-0.408935
139.5	4000000.	102.	1.500	0.33	-0.481486
139.5	4000000.	9.	2.000	0.35	-0.455932
139.5	4000000.	28.	2.000	0.39	-0.408935
139.5	4000000.	15.	2.000	0.37	-0.431798
139.5	4000000.	16.	2.000	0.37	-0.431798
139.5	4000000.	12.	2.000	0.36	-0.443697
139.5	4000000.	14.	2.000	0.37	-0.431798
139.5	4000000.	45.	2.000	0.40	-0.397940
135.4	4000000.	107.	7.000	1.53	0.184691
135.4	4000000.	30.	8.000	1.56	0.193125
135.4	4000000.	83.	7.000	1.49	0.173186
135.4	4000000.	58.	7.000	1.45	0.161368
135.4	4000000.	77.	7.000	1.48	0.170262
135.4	4000000.	26.	7.000	1.35	0.130334
135.4	4000000.	92.	7.000	1.51	0.178977
135.4	4000000.	58.	7.000	1.45	0.161368
135.4	4000000.	57.	7.000	1.44	0.158362
135.4	4000000.	32.	7.000	1.37	0.136721

TABLE 5 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
135.4	3670000.	32.	7.000	1.38	0.139879
135.4	3670000.	92.	7.000	1.52	0.181844
135.4	3670000.	48.	7.000	1.43	0.155336
135.4	3670000.	49.	7.000	1.44	0.158362
135.4	3670000.	95.	7.000	1.53	0.184691

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
139.0	-0.302978	16.392	-0.120

X VALUE	STD. DEV.	ADJ. STD. DEV.
141.8	0.009	0.013
139.5	0.009	0.009
135.4	0.009	0.015
141.8	0.009	0.013

DATA POINTS	X	Y	95% Confidence interval
	141.8	-0.624	-0.650....-0.598
	139.5	-0.358	-0.376....-0.340
	135.4	+0.144	+0.114....+0.174

The coefficient of correlation is -0.980.

The T-distribution value is 3.500.

Significant beyond .001 level of significance.

FIGURE 4. Thermal destruction curve of E. coli in 0.85% sodium chloride pH 7.0.

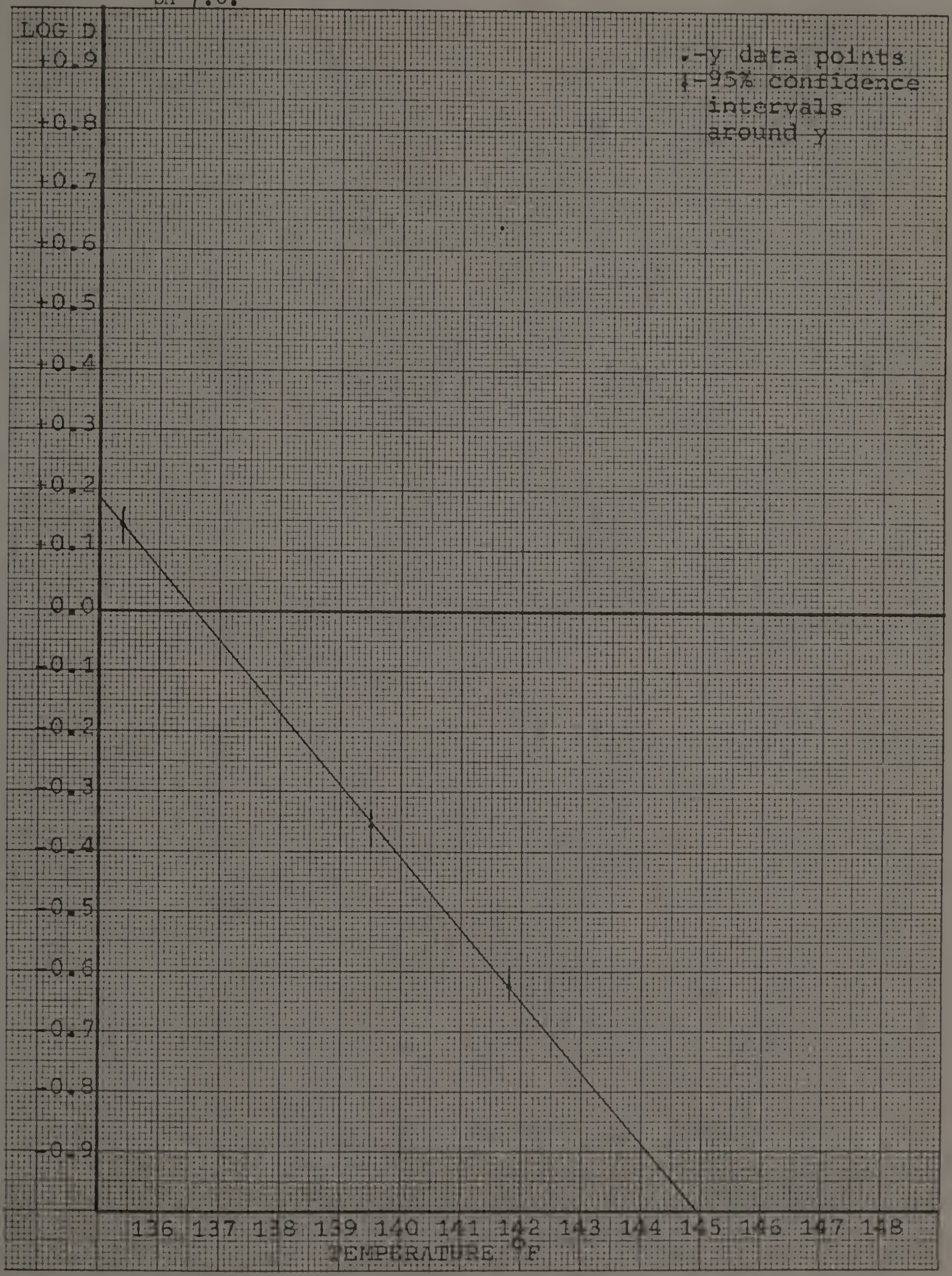


TABLE 6. Thermal destruction calculations for E. coli in 8.5% sodium chloride pH 5.4.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
143.7	4330000.	47.	4.500	0.91	-0.040959
143.7	4330000.	64.	4.000	0.83	-0.080922
143.7	4330000.	27.	4.500	0.86	-0.065502
143.7	4330000.	83.	4.000	0.85	-0.070581
143.7	4330000.	25.	4.500	0.86	-0.065502
143.7	4330000.	34.	4.500	0.88	-0.055517
143.7	4330000.	29.	4.500	0.87	-0.060481
143.7	4330000.	12.	4.500	0.81	-0.091515
143.7	4330000.	48.	4.000	0.81	-0.091515
143.7	4330000.	92.	4.000	0.86	-0.065502
141.8	4000000.	97.	6.500	1.41	0.149219
141.8	4000000.	74.	6.000	1.27	0.103804
141.8	4000000.	55.	6.000	1.23	0.089905
141.8	4000000.	42.	6.000	1.21	0.082785
141.8	4000000.	19.	6.500	1.22	0.086360
141.8	4000000.	26.	6.500	1.25	0.096910
141.8	4000000.	64.	7.000	1.46	0.164353
141.8	4000000.	44.	7.000	1.41	0.149219
141.8	4000000.	80.	7.000	1.49	0.173186
141.8	4000000.	28.	7.000	1.36	0.133539
139.5	4330000.	40.	10.500	2.09	0.320146
139.5	4330000.	31.	10.500	2.04	0.309630
139.5	4330000.	50.	10.500	2.13	0.328380

TABLE 6 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
139.5	4330000.	15.	10.000	1.83	0.262451
139.5	4330000.	24.	10.500	2.00	0.301030
139.5	4330000.	34.	10.000	1.96	0.292256
139.5	4330000.	26.	10.000	1.92	0.283301
139.5	4330000.	29.	10.000	1.93	0.285557
139.5	5330000.	45.	10.000	1.97	0.294466
139.5	5330000.	63.	10.000	1.97	0.294466
139.5	5330000.	77.	10.000	2.07	0.315970
139.5	5330000.	130.	10.000	2.17	0.336460
139.5	5330000.	90.	10.000	2.10	0.322219
139.5	5330000.	30.	10.000	1.90	0.278754
139.5	5330000.	60.	10.000	2.02	0.305351
139.5	5330000.	32.	10.000	1.92	0.283301
139.5	5330000.	46.	10.000	1.97	0.294466
139.5	5330000.	74.	10.000	2.06	0.313867

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
141.2	0.157273	12.454	-0.087

X VALUE	STD. DEV.	ADJ. STD. DEV.
143.7	0.004	0.007
141.8	0.004	0.004
139.5	0.004	0.006

TABLE 6 continued

DATA POINTS	X	Y	95% Confidence interval
	143.7	-0.479	-0.491....-0.465
	141.8	-0.100	-0.108....-0.002
	139.5	+0.317	+0.305....+0.329

The coefficient of correlation is -0.987.

The T-distribution value is 2.981.

Significant beyond .01 level of significance.

FIGURE 5. Thermal destruction curve of E. coli in 8.5% sodium chloride pH 5.4.

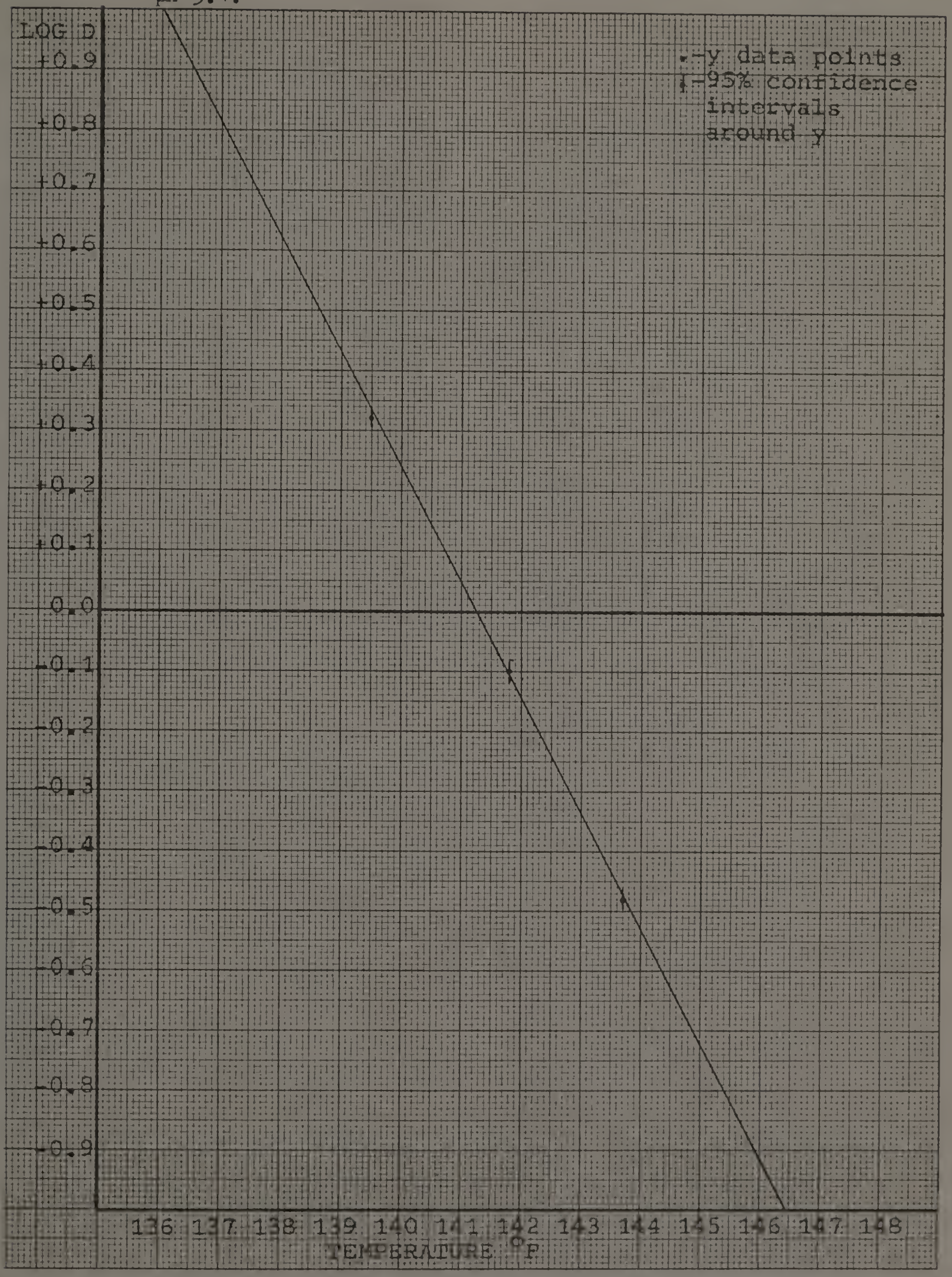


TABLE 7. Thermal destruction calculations for E. coli in 8.5% sodium chloride pH 7.0.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
143.7	4000000.	14.	2.000	0.37	-0.431798
143.7	4000000.	40.	2.000	0.40	-0.397940
143.7	4000000.	127.	1.500	0.33	-0.481486
143.7	4000000.	140.	1.500	0.34	-0.468521
143.7	4000000.	28.	2.500	0.48	-0.318759
143.7	4000000.	22.	2.500	0.48	-0.318759
143.7	4000000.	16.	2.000	0.37	-0.431798
143.7	4000000.	13.	2.000	0.36	-0.443697
143.7	4000000.	17.	2.000	0.37	-0.431798
143.7	4000000.	12.	2.000	0.36	-0.443697
141.8	3670000.	54.	3.000	0.62	-0.207608
141.8	3670000.	30.	3.000	0.59	-0.229148
141.8	3670000.	37.	3.000	0.60	-0.221849
141.8	3670000.	94.	3.000	0.65	-0.187087
141.8	3670000.	34.	3.000	0.60	-0.221849
141.8	3670000.	40.	3.000	0.60	-0.221849
141.8	3670000.	120.	3.000	0.67	-0.173925
141.8	3670000.	88.	3.000	0.65	-0.187087
141.8	3670000.	22.	3.500	0.67	-0.173925
141.8	3670000.	24.	3.500	0.68	-0.167491
141.8	3670000.	82.	3.000	0.65	-0.187087
141.8	3670000.	76.	3.000	0.64	-0.193820
139.5	5330000.	44.	5.500	1.08	0.033424

TABLE 7 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
139.5	5330000.	39.	5.000	0.97	-0.013228
139.5	5330000.	74.	5.500	1.13	0.053078
139.5	5330000.	84.	5.500	1.15	0.060698
139.5	5330000.	33.	5.500	1.06	0.025306
139.5	5330000.	23.	5.500	1.03	0.012837
139.5	5330000.	54.	5.500	1.10	0.401393
139.5	5330000.	13.	5.500	0.98	-0.008774
139.5	5330000.	17.	5.500	1.00	0.000000
139.5	5330000.	33.	5.500	1.06	0.025306

X VALUE MEAN	LOG(D) VALUE MEAN.	A	B
141.7	-0.197217	14.596	-0.104

X VALUE	STD. DEV.	ADJ. STD. DEV.
139.5	0.007	0.011
141.8	0.007	0.007
143.7	0.007	0.010

DATA POINTS	X	Y	95% Confidence interval
	143.7	-0.349	-0.371....-0.321
	141.8	-0.151	-0.165....-0.137
	139.5	+0.088	+0.068....+0.108

TABLE 7 continued

The coefficient of correlation is -0.979.

The T-distribution value is 2.709.

Significant beyond 0.02 level of significance.

FIGURE 6. Thermal destruction curve of E. coli in 8.5% sodium chloride pH 7.0.

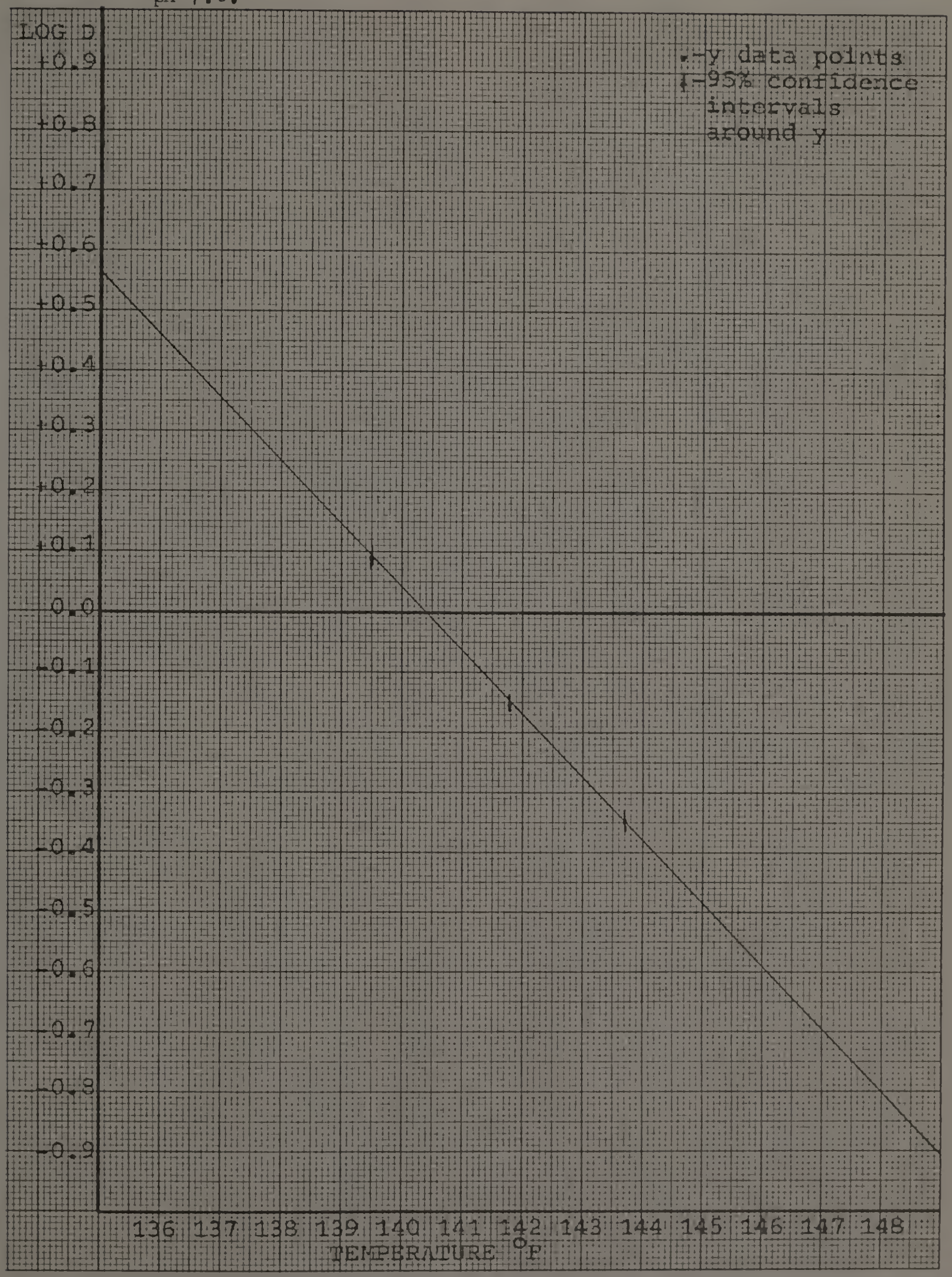


TABLE 8. Thermal destruction calculations for E. coli in 39.6% glycerol pH 7.0.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
147.6	1400000.	6.	3.0	0.56	-0.251812
147.6	1400000.	26.	3.0	0.63	-0.200659
147.6	1400000.	16.	3.0	0.61	-0.214670
147.6	1400000.	120.	3.0	0.74	-0.130768
147.6	1400000.	144.	3.0	0.75	-0.124939
147.6	1400000.	30.	3.0	0.64	-0.193820
147.6	1400000.	35.	3.0	0.65	-0.187087
147.6	1400000.	58.	3.0	0.68	-0.167491
145.7	1400000.	56.	4.0	0.91	-0.040959
145.7	1400000.	20.	5.0	1.03	0.012837
145.7	1400000.	10.	4.0	0.78	-0.107905
145.7	1400000.	56.	4.0	0.91	-0.040959
145.7	1400000.	353.	3.0	0.83	-0.080922
145.7	1400000.	28.	5.0	1.06	0.025306
145.7	1400000.	55.	4.0	0.91	-0.040959
145.7	1400000.	50.	4.0	0.90	-0.045757
145.7	1400000.	84.	4.0	0.95	-0.022276
145.7	1400000.	85.	4.0	0.95	-0.022276
143.7	1100000.	65.	10.0	2.36	0.372912
143.7	1100000.	60.	10.0	2.35	0.371068
143.7	1100000.	80.	10.0	2.42	0.383815
143.7	1500000.	33.	11.0	2.36	0.372912
143.7	1500000.	58.	10.0	2.27	0.356026

TABLE 8 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
143.7	1500000.	130.	10.0	2.46	0.390935

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
145.8	0.017190	20.451	-0.140

X VALUE	STD. DEV.	ADJ. STD. DEV.
147.6	0.015	0.023
145.7	0.015	0.015
143.7	0.015	0.026

DATA POINTS	X	Y	95% Confidence interval
	147.6	-0.21	-0.16....-0.26
	145.7	+0.05	+0.02....+0.08
	143.7	+0.33	+0.28....+0.38

The coefficient of correlation is -0.948.

The T-distribution value is 2.282.

Significant beyond 0.05 level of significanco.

FIGURE 7. Thermal destruction curve of E. coli in 39.6% glycerol pH 7.0.

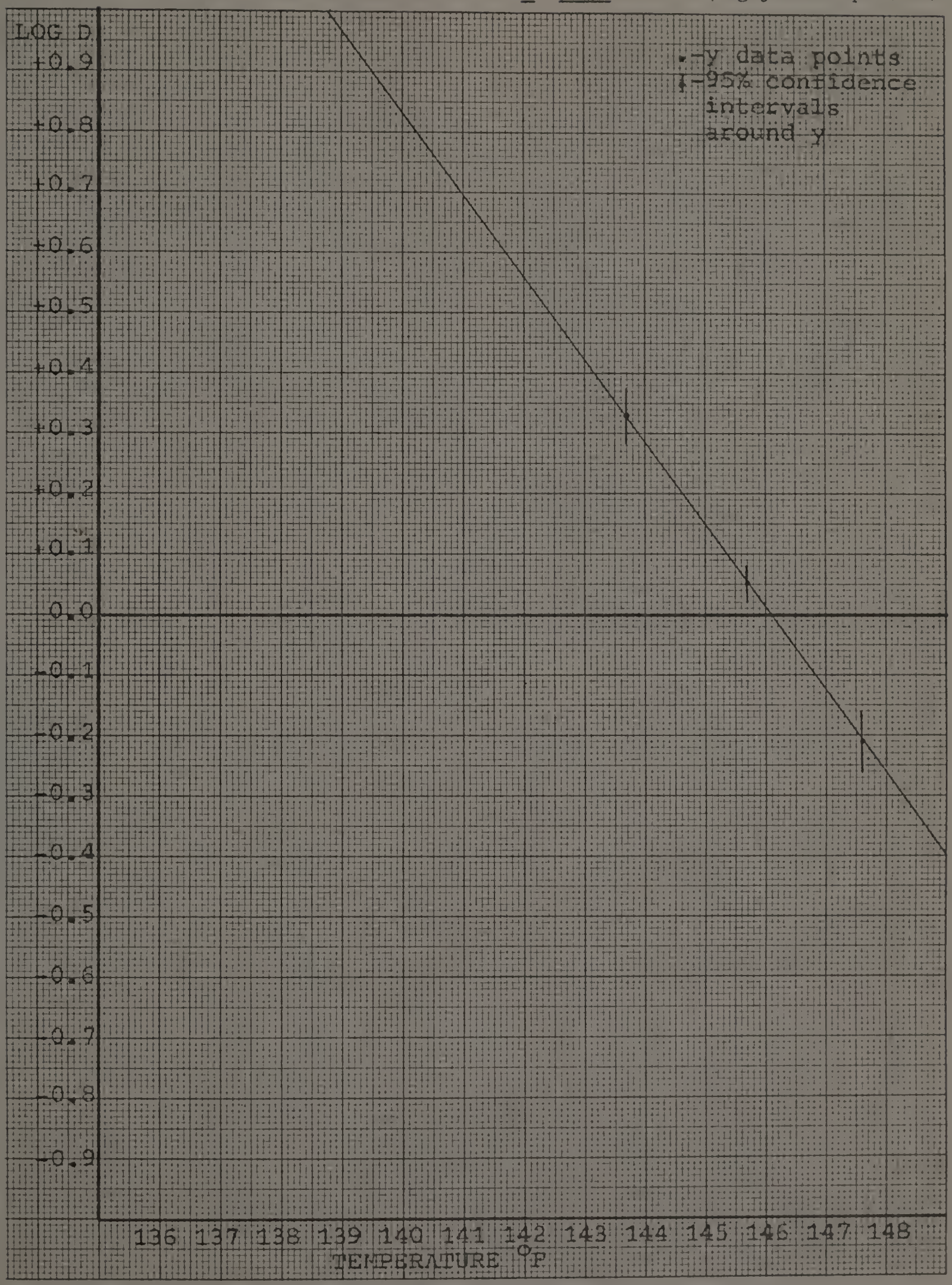


TABLE 9. Thermal destruction calculations for E. coli in 15.4% glycerol pH 7.0.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
143.7	4300000.	20.	2.5	0.47	-0.327902
143.7	4300000.	34.	2.5	0.49	-0.309804
143.7	4300000.	37.	2.5	0.49	-0.309804
143.7	4300000.	58.	2.5	0.51	-0.292430
143.7	4300000.	20.	2.5	0.47	-0.327902
143.7	4300000.	51.	2.5	0.51	-0.292430
143.7	4300000.	29.	2.5	0.48	-0.318759
143.7	4300000.	50.	2.5	0.51	-0.292430
143.7	4300000.	52.	2.5	0.51	-0.292430
143.7	4300000.	41.	2.5	0.50	-0.301030
141.8	4000000.	66.	3.5	0.73	-0.136677
141.8	4000000.	52.	3.5	0.72	-0.142668
141.8	4000000.	30.	3.5	0.68	-0.167491
141.8	4000000.	46.	3.5	0.71	-0.148742
141.8	4000000.	93.	3.5	0.76	-0.119186
141.8	4000000.	36.	3.5	0.69	-0.161151
141.8	4000000.	90.	3.5	0.75	-0.124939
141.8	4000000.	46.	3.5	0.71	-0.148742
141.8	4000000.	154.	3.5	0.79	-0.102373
141.8	4000000.	64.	3.5	0.73	-0.136677
139.5	3000000.	17.	5.0	0.95	-0.022276
139.5	3000000.	19.	5.0	0.96	-0.017729
139.5	3000000.	54.	5.0	1.05	0.021189

TABLE 9 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
139.5	3000000.	60.	5.0	1.06	0.025306
139.5	3000000.	89.	5.0	1.10	0.041393
139.5	3000000.	88.	5.0	1.10	0.041393
139.5	3000000.	37.	5.0	1.02	0.008600
139.5	3000000.	19.	5.0	0.96	-0.017729
139.5	3000000.	21.	5.0	0.97	-0.013228
139.5	3000000.	50.	5.0	1.05	0.021189

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
141.7	-0.145515	10.437	-0.075

X VALUE	STD. DEV.	ADJ. STD. DEV.
143.7	0.004	0.007
141.8	0.004	0.004
139.5	0.004	0.007

DATA POINTS	X	Y	95% Confidence interval
	143.7	-0.34	-0.33....-0.36
	141.8	-0.20	-0.19....-0.21
	139.5	-0.03	-0.01....-0.04

The coefficient of correlation is -0.935.

The T-distribution value is 2.626.

Significant beyond 0.02 level of significance.

FIGURE 8. Thermal destruction curve of E. coli in 15.4% glycerol pH 7.0.

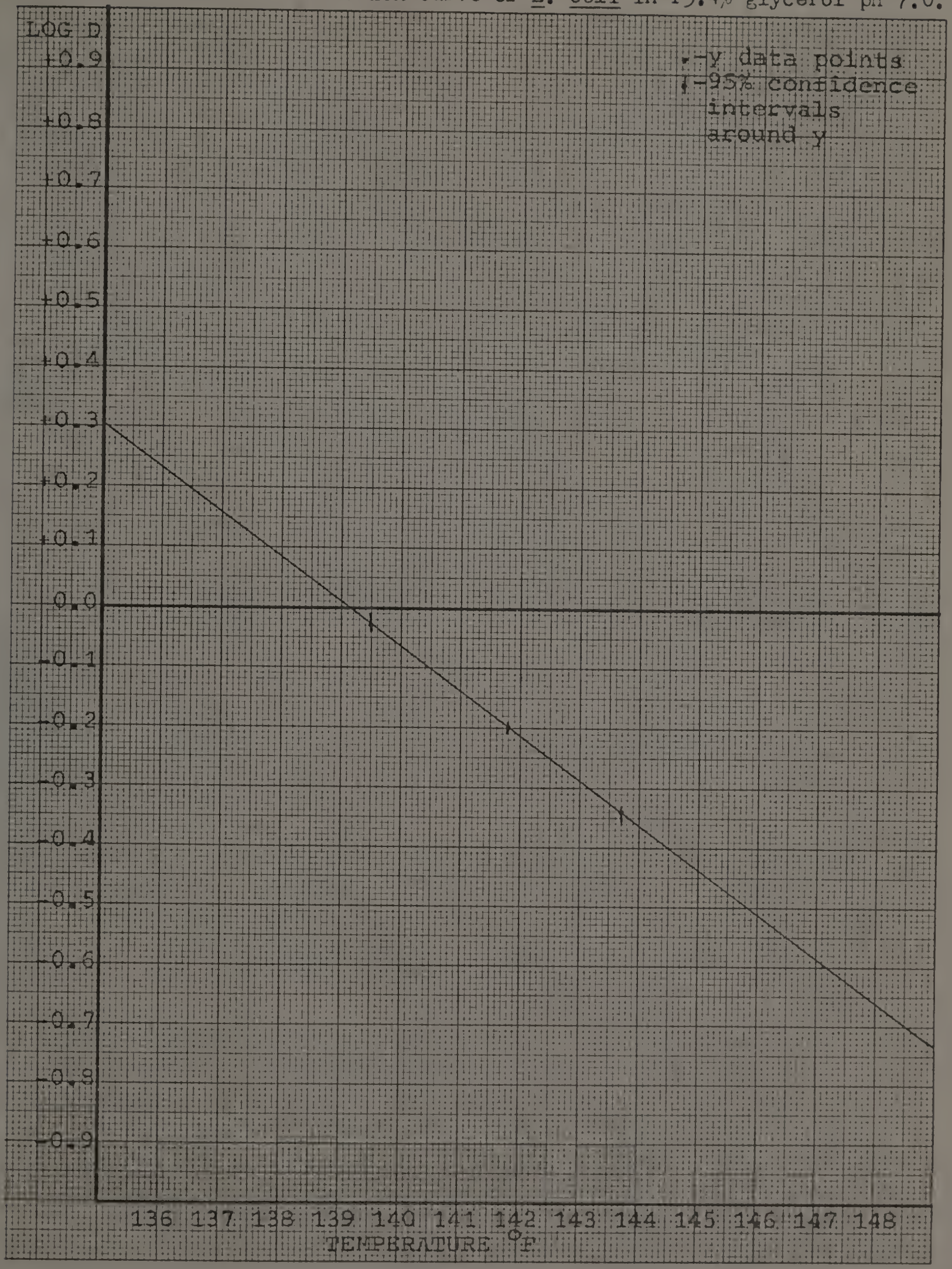


TABLE 10. Thermal destruction calculations for E. coli in 0.01 M phosphate buffer pH 7.0.

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
143.7	1200000.	180.	1.5	0.39	-0.408935
143.7	1200000.	37.	1.5	0.33	-0.481486
143.7	1200000.	75.	1.5	0.36	-0.443687
143.7	1600000.	10.	1.5	0.29	-0.537602
143.7	1600000.	80.	1.5	0.35	-0.455932
143.7	1300000.	96.	1.5	0.36	-0.443697
143.7	1300000.	148.	1.5	0.38	-0.420216
143.7	1300000.	156.	1.5	0.38	-0.420216
143.7	1300000.	15.	2.0	0.41	-0.387216
143.7	1300000.	12.	2.0	0.40	-0.397940
141.8	1900000.	53.	2.0	0.44	-0.356547
141.8	1900000.	180.	2.0	0.50	-0.301030
141.8	1900000.	36.	2.0	0.42	-0.376751
141.8	1900000.	104.	2.0	0.47	-0.327902
141.8	1900000.	75.	2.5	0.57	-0.244125
141.8	1900000.	73.	2.0	0.45	-0.346787
141.8	1700000.	120.	2.5	0.60	-0.221849
141.8	1700000.	23.	2.0	0.41	-0.387216
141.8	1700000.	33.	2.0	0.42	-0.376751
141.8	1700000.	12.	2.0	0.39	-0.408935
135.4	1500000.	24.	8.0	1.67	0.222716
135.4	1500000.	45.	8.0	1.77	0.247973
135.4	1500000.	32.	8.0	1.71	0.232996

TABLE 10 continued

TEMPERATURE	INITIAL COUNT	FINAL COUNT	TIME	D VALUE	LOG(D)
135.4	1500000.	19.	8.0	1.63	0.212188
135.4	1500000.	26.	8.0	1.68	0.225309
135.4	1500000.	47.	8.0	1.78	0.250420
135.4	1500000.	64.	8.0	1.83	0.262451
135.4	1900000.	45.	8.0	1.73	0.238046
135.4	1900000.	30.	8.0	1.67	0.222716
135.4	1900000.	23.	8.0	1.63	0.212188

X VALUE MEAN	LOG(D) VALUE MEAN	A	B
140.3	-0.180594	11.458	-0.083

X VALUE	STD. DEV.	ADJ. STD. DEV.
143.7	0.009	0.013
141.8	0.009	0.010
135.4	0.009	0.016

DATA POINTS	X	Y	95% Confidence interval
	143.7	-0.47	-0.44....-0.50
	141.8	-0.31	-0.29....-0.33
	135.4	+0.22	+0.19....+0.25

The coefficient of correlation is -0.987.

The T-distribution value is 2.628.

Significant beyond 0.02 level of significance.

FIGURE 9. Thermal destruction curve of E. coli in 0.01 M phosphate buffer pH 7.0.

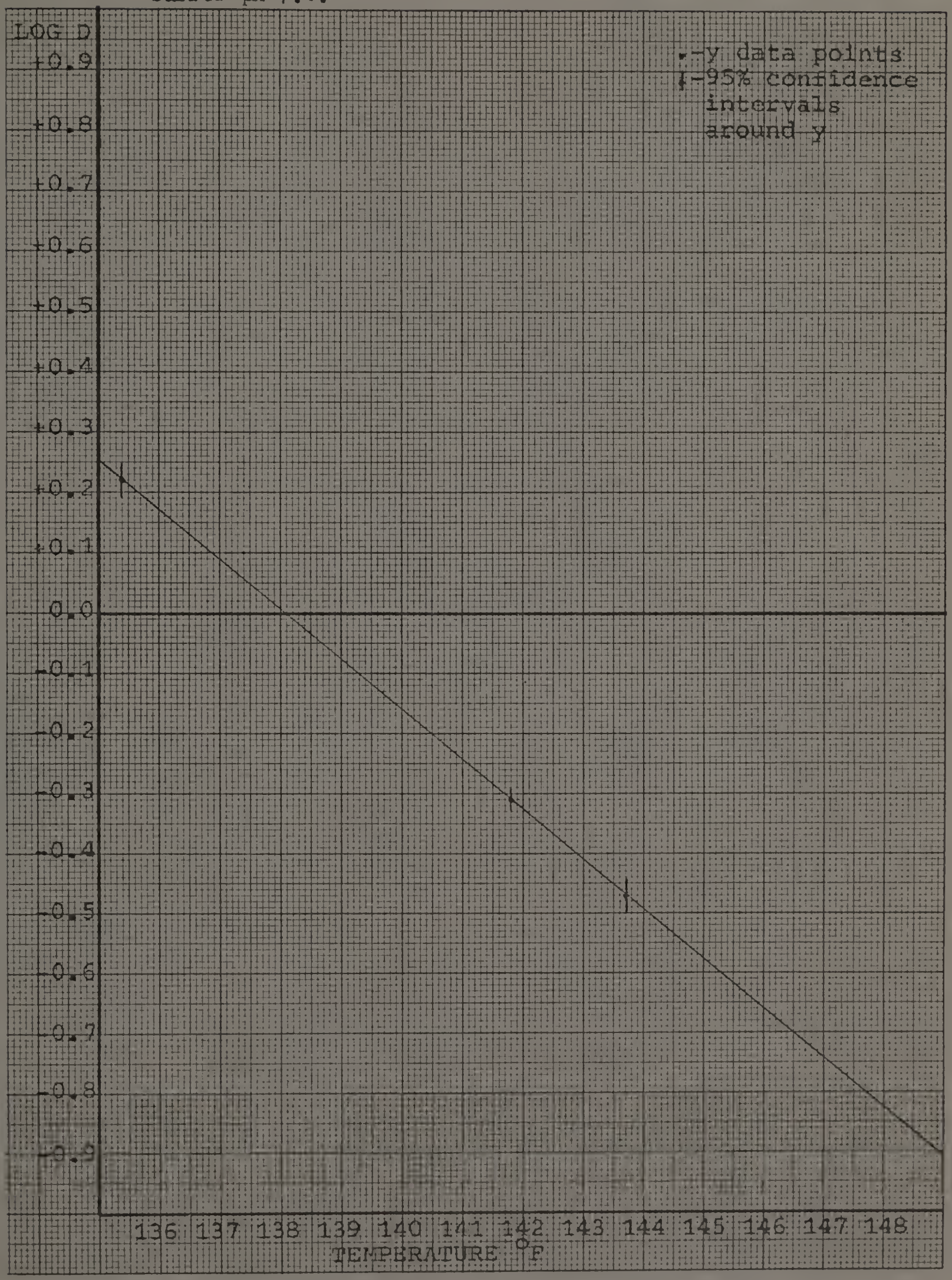


TABLE 11. Thermal resistance of *E. coli* in various menstra at 143.7 F.

	MEAN LOG(D)	VARIANCE	STANDARD DEVIATION	SLOPE OF REGRESSION LINES THROUGH 143.7 F
1. 0.01M phosphate buffer pH 7.0	-0.440	0.0020	0.045	-0.083
2. 39.6% glycerol in 0.01M phosphate buffer pH 7.0	+0.375	0.0001	0.012	-0.140
3. 15.4% glycerol in 0.01M phosphate buffer pH 7.0	-0.306	0.0002	0.015	-0.075
4. 0.01M magnesium acetate in 0.01M phosphate buffer pH 7.0	-0.612	0.0003	0.018	-0.091
5. 0.01M citric acid in 0.01M phosphate buffer pH 7.0	-0.457	0.003	0.018	-0.097
6. 0.85% sodium chloride in 0.01M phosphate buffer pH 5.4	-0.563	0.0029	0.053	-0.109
7. 8.5% sodium chloride in 0.01M phosphate buffer pH 5.4	-0.069	0.003	0.016	-0.087
8. 8.5% sodium chloride in 0.01M phosphate buffer pH 7.0	-0.417	0.0032	0.056	-0.104
9. 0.85% sodium chloride in 0.01M phosphate buffer pH 7.0	-0.875	0.003	0.018	-0.120

TABLE 12. Analysis of variance of E. coli in various menstra at 143.7 F.

SETS 1,2,3	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE
Between	5	8.941	1.788
Within	46	0.048	0.001
Total	51	8.989	

F 5,46 = 1,788

SETS 4-9	DEGREE OF FREEDOM	SUM OF SQUARE	MEAN SQUARE
Between	4	2.134	0.533
Within	62	0.112	0.002
Total	66	2.246	

F 4,62 = 1,066

buffer pH 7.0 was found to be the most protective while the 0.85% sodium chloride in 0.01 M phosphate buffer pH 7.0 was the least protective.

These results also indicate that there may be an indirect relationship between thermal resistance and pH in that as the pH of 0.85% sodium chloride was decreased from 7.0 to 5.4 the log D values increased from -0.875 to -0.563. This is further substantiated by the results obtained with 8.5% sodium chloride. Similar experiments conducted by Libbey,[45] employing a phosphate buffer menstrum, demonstrated that the thermal resistance was higher for E. coli in a slightly acid menstra at pH 6.0 than at pH 7.0.

Figures 9 and 10 illustrate water activity affects the thermal resistance of microbial cells in that the resistance increases with decreasing humidity. The water activity in the systems employed are directly related to the amount of glycerol present, i.e., the more glycerol the less the water activity. These figures show that the slope of the destruction curve for 39.6% glycerol at 143.7 F is almost twice that which contains 15.4% glycerol. The heat mechanism by which cells have been related to water activity include inactivation of nucleic acids, enzymes or other essential cellular proteins.

Table 12 shows the analysis of variance of the log D values at 143.7 F in the presence of and absence of 39.6% glycerol in 0.01 M phosphate buffer pH 7.0. In both sets of data the F ratios, a comparison of between variability to within variability, were significant beyond the 0.01 level. Thus, all the treatments employing various menstra produced significantly different thermal resistance levels.

The mechanisms by which the death of a cell occurred in various

TABLE 13. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 50 C in 0.01 M citric acid pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.366	5.5	12
3.5	8	.602	7	16
10.0	7	.704	10	18
20.0	7	.762	15	22.5
40.0	6	.985	22	28
60.0	5	1.32	28	34.5
90.0	4	1.37	35	35
120.0	3	1.60	37.5	43.5

TABLE 14. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 60 C in 0.01 M citric acid pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.274	.5	11.8
2.0	6	.292	2.0	10.6
5.0	2	.347	5.5	17.3
10.0	1	.366	7.0	11.8
20.0	0	.450	9.0	16.5
40.0	0	.618	12.0	19.5
60.0	0	.780	15.0	22.5
90.0	0	1.08	22.5	27.0
120.0	0	1.39	26.5	29.2

TABLE 15. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 50 C in 0.01 M magnesium acetate pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.492	3.0	12.5
3.5	8	.523	5	13.5
10.0	7	.621	8.5	16.5
20.0	6	.700	10	17.5
40.0	6	.860	15	24
60.0	5	.970	18	26
90.0	4	.975	19.7	27
120.0	4	1.00	20.4	28

TABLE 16. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 60 C in 0.01 M magnesium acetate pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.394	2	8.0
2.0	2	.398	3.5	9.0
5.0	2	.448	6	10.5
10.0	0	.494	7.5	11
20.0	0	.598	9.2	13.5
40.0	0	.648	12.5	15
60.0	0	.682	13.5	15
90.0	0	.748	15	17
120.0	0	.818	16	18

TABLE 17. The effect of temperature on the viability of and release of intracellular constituents from suspensions of E. coli at 50 C in 8.5% sodium chloride pH 7.0.

Time/ min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.292	6.0	12.5
2.0	8	.317	7.0	12.6
5.0	8	.338	9.8	13.0
10.0	7	.352	10.0	13.0
20.0	7	.415	12.2	13.3
40.0	6	.452	13.0	18.5
60.0	6	.450	13.5	21.0
90.0	5	.472	14.0	21.6
120.0	5	.492	14.0	23.2

TABLE 18. The effect of temperature on the viability of and release of intracellular constituents from suspensions of E. coli at 60 C in 8.5% sodium chloride pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.341	4.0	17
2.0	6	.460	4.5	18
5.0	4	.480	8.0	22
10.0	0	.501	10.5	23
20.0	0	.520	16.5	27
40.0	0	.541	17.0	30
60.0	0	.515	19.8	32
90.0	0	.610	22.0	36
120.0	0	.660	26.5	40

TABLE 19. The effect of temperature on the viability of and release of intracellular constituents from suspensions of E. coli at 50 C in 0.01 M 0.85% sodium chloride pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.167	4	15.0
2.0	8	.395	8.5	17.3
5.0	7	.955	9.5	27.9
10.0	7	1.32	12.4	36.0
20.0	6	1.70	18.7	43.3
40.0	4	1.95	24	47.0
60.0	4	2.0	29	52.5
90.0	2	2.0	37	53.8
120.0	0	2.00	46	55.7

TABLE 20. The effect of temperature on the viability of and release of intracellular constituents from suspensions of E. coli at 60 C in 0.01 M 0.85% sodium chloride pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.162	2.0	10.0
5.0	1	.585	8.1	16.5
10.0	0	.680	11.3	18.3
20.0	0	.725	14.6	20.3
40.0	0	.845	19.0	20.5
60.0	0	.960	22.4	24.5
90.0	0	1.15	23.6	26.3
120.0	0	1.33	24.0	29.8

TABLE 21. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 50 C in 15.4% glycerol pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.330	1.1	11.3
5.0	8	.454	3.5	12.5
10.0	7	.450	5.5	13.3
20.0	7	.482	7.4	14.0
40.0	6	.567	9.8	16.3
60.0	6	.626	10.75	17.5
90.0	4	.678	13.75	18.3
120.0	3	.772	15.8	20.3

TABLE 22. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 60 C in 15.4% glycerol pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.214	2	16.5
2.0	7	.366	11.2	17.0
5.0	1	.461	10.0	18.3
10.0	0	.554	12.5	18.5
20.0	0	.652	16	24.5
40.0	0	.785	19.5	22.8
60.0	0	.999	24	25.3
90.0	0	1.27	30	25.5
120.0	0	1.47	32	29.0

TABLE 23. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 50 C in 0.01 M phosphate pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.276	1.7	4.3
2.0	8	.638	2.0	17.3
5.0	7	.890	2.5	21.9
10.0	7	1.02	5.0	26.3
20.0	6	1.13	11.0	24.5
40.0	5	1.19	11.5	26.0
60.0	5	1.24	13.5	29.2
90.0	4	1.33	16.5	30.8
120.0	4	1.30	17.5	32.7

TABLE 24. The effect of temperature on the viability of and release of intracellular constituents from suspensions of *E. coli* at 60 C in 0.01 M phosphate pH 7.0.

Time/ Min.	log Viable Cells/ml	OD at 260 mu	Ribose Concentration ug/ml	Protein Concentration ug/ml
.5	8	.226	1.0	12.5
2.0	7	.264	3.0	14.0
5.0	5	.298	4.5	15.2
10.0	1	.300	5.5	17.0
20.0	0	.364	8.0	19.3
40.0	0	.472	9.5	17.9
60.0	0	.572	12.0	18.5
90.0	0	.655	15	19.3
120.0	0	.785	16	18.3

menstra were also investigated. Standard analysis for cellular components and viable cells was conducted on all heated menstra. The data presented in Tables 13-24 are cumulative averages of four trials performed during an eight week period. The cellular contents excreted during heating were protein, ribose and a UV absorbing substance. Analysis for the latter, after heat treatment, showed that maximum absorption occurred at 255-260 mu, with base ratios of 250:260 and 280:260 respectively of 1:1. However, all unheated samples stored at room temperature had no significant absorption (Appendix Part III).

Cells of E. coli were ruptured by sonic vibration and the presence of DNA was established. However, this cellular component could not be found in the supernatant of the heated cells.

The leakage of cell constituents, other than DNA, which occurred as a result of heating indicates that a permeability control mechanism was involved, and such a mechanism is located at the cytoplasmic membrane.

The data presented in Tables 13-24 indicates a relationship between the numbers of survivors and the amount of leakage products and that this is dependent upon the nature of the suspending medium.

These results suggest a relationship between the rate of death and RNA degradation occurring at 50 C, the lower temperature studied. As illustrated in Tables 13 and 15 the cells that were suspended in magnesium yielded a low number of survivors, with a corresponding low analysis for ribose, whereas those suspended in citrate yielded an even lower number of survivors with an increase in ribose present in the supernatant. The latter confirms results reported by Chao[19] and Wade[81] that citrate accelerates ribosomal breakdown. The most lethal of all the menstra

tested was found to be 0.85% sodium chloride pH 7.0 which also produced the highest concentration of leakage products, (Tables 19 and 20). These data demonstrate that the loss in viable numbers during heating at 50 C is a consequence of the nature of the suspending menstra.

Observations suggest that the presence of leakage products can be determined by UV absorption. All extracts were treated with 10% cold TCA and the soluble fraction did not contain any peak absorption from 250-270 mu.

The pH of the E. coli suspensions were tested at the end of each heating treatment, the maximum pH variation was approximately ±0.2 pH units. Thus, it is unlikely that pH changes in the external environment play any significant part in the thermal destruction process.

DISCUSSION

The biological injury that results when microorganisms are heated is manifested by losses in viable numbers which follow a logarithmic order as exemplified by the data and the leakage of intracellular constituents. Such results can be attributed to either lysis or rupturing of the cell wall or some change in the cell membrane, the structures responsible for maintaining the form and integrity of the integral constituents.

The absence of any detectable deoxyribose in the cell extracts would also indicate that lysis did not occur. Cells ruptured by sonic vibration showed the presence of this carbohydrate which is attributed to DNA. The presence of DNA would indicate a structure with no limiting membrane or a membrane which is ruptured at the time of lysis. If heated cells are ruptured then they, too, should liberate DNA. None was detected in this study. Therefore, such results would refute any theory of death by "Mechanical Crushing".

The effect of heat apparently lies in some alteration of the cell membrane. The cell membrane not only preserves the integrity of the cell by preventing leakage of the cytoplasmic constituents into the environment, but is also concerned with the transport of nutrients into the cell. Any agent or stress that damages this semi-permable membrane by altering its physico-chemical structure would cause disorganization of cellular function. When its selective properties are lost components leak out of the cell resulting in injury or death. These factors differed at the two different temperatures studied.

The data from all of the menstra, except 8.5% sodium chloride and 15.4% glycerol, shows that the leakage of 260 mu absorbing materials from the cells appeared to have a direct correlation to the death rate at 50 C. At 60 C a different response pattern was noted in that a maximum level of leakage was rapidly reached and maintained while the death rate continued to follow a linear response. More specifically cells heated at 50 C in 15.4% glycerol and 8.5% sodium chloride exhibited less leakage than the other menstra used. On the other hand, at 60 C it was found that the amount of leakage was greater for 8.5% sodium chloride and 15.4% glycerol as demonstrated by spectrographic and ribose determinations.

Therefore, the data suggested that there is a greater degree of RNA degradation at 60 C in cells heated in 15.4% glycerol and 8.5% sodium chloride than those in the other menstra. The data for these two menstra indicates the presence of a greater amount of pooled RNA at 60 C than at 50 C. Also, the UV absorption and ribose determinations indicate a decrease in the rate of protein coagulation in cells heated in 15.4% glycerol and 8.5% sodium chloride at 60 C. This suggests that leakage at 60 C is not a primary effect of moist heat on E. coli. This phenomenom may be the result of the intracellular coagulation of protein combining with RNA like material preventing further leakage when cells are heated at 60 C in non protective menstra. These data are in accord with that of Allwood and Russell[2,3,4,5] who demonstrated that cells of E. coli heated at 60 C developed a coarsely granular appearance, presumed to be protein coagulation.

The protective action of glycerol in this study is compatible

with the results obtained by other investigators.[4,5,52,64,68] The protective action is attributed to the ability of glycerol to stabilize permeability.

The losses in number in menstra that should have provided protection might be due to the washing process in the preparation of the cell suspension. This study used sterile distilled water. Strange and Shon[74] reported that washing with saline desorbs magnesium ions from the cells which increased sensitivity to thermal stress.

However, the cells that were suspended in magnesium did not exhibit the greatest resistance, yet the concentrations of ribose recorded were low. The activity of magnesium appears to lie in its stabilization of the ribosome and ribo-nucleo-protein as shown by Lederberg[42] and Weibull.[83] Elson and Tal[25] showed that when these ions are replaced or removed by monovalent cations, the ribonucleic particles undergo a series of dissociations first reversible, then irreversible.

This affect of the stability of the ribosome can be seen both with magnesium, which decreased the amount of ribose, and with citrate, which has a reverse effect in accelerating ribosomal breakdown.

It is necessary to try to explain the decreased resistance of cells heated in 0.85% sodium chloride pH 7.0. First, it was determined by Mitchell and Moyle[49] that the cell membrane is permeable to sodium ions. This would explain the breakdown of the ribonucleoprotein and the leakage of the intracellular products. Elson[25] has shown that the addition of sodium chloride to isolated RNA affected a separation of the molecule. Secondly, sodium is necessary in water metabolism of cells. It controls the movement of water and sodium

intake, thus affecting or controlling hydration.

The data presented in the thermal resistance of E. coli and the response of this organism in suspensions suggest that the cause of the phenomenom is an increase in cell permeability. Substances that stabilize permeability provided protection while those that did not showed an increase in leakage products. Although there is this release of materials from cells, it does not necessarily follow that death occurs. Rotman[64] has shown that cells can lose as much as 80% of their RNA and still remain viable.

The characteristics of the UV absorbing material obtained in this study appear to be the same as those reported by Allwood and Russell:[2,3,4,5] the UV spectra obtained indicated maximum absorption from 255-260 mu indicative of RNA.

The base ratios at 250:260 and 280:260 were approximately 1:1 which further supports this assumption as stated by Strange and Shon.[74] Also, the absence of the characteristic absorption peak of 250-270 mu in the TCA soluble fraction indicates that this UV absorbing material is RNA.

The material that is apparently considered to be protein might be peptide. Absorption peaks were absent in the region of 280 mu. It is possible that no protein is present or the protein lacks the amino acids tyrosine and tryptophane that give the characteristic absorption peak. Protein was detected colorimetrically by the Folin reagent which is 10-20 times more sensitive than absorption at 280 mu which could account for the detection of protein colorimetrically, but not by absorption.

However, the fact that protein concentrations decrease at 60 C

indicates the possibility of intracellular protein coagulation enmeshing RNA like material, therefore preventing further leakage, a phenomenom which does not occur at lower temperatures, or in 15.4% glycerol and 8.5% sodium chloride pH 7.0.

Our knowledge of the primary damage induced by moist heat in nonsporing bacteria remains incomplete. It has been indicated that RNA is degraded at these temperatures and precedes leakage from the acid soluble nucleotide pool. Moreover, Sorgin and Ordal[71] implicated ribosomal RNA as the lesion site in thermally injured Staph. aureus. Thus, the finding in this report needs to be considered in relation to these observations.

Even today the statement made by Schmidt[67] in 1954 is still pertinent:

> The search for an investigation of those factors and conditions which may influence the thermal resistance of microorganisms would seem an unending task, but one which is assuredly profitable either in providing explanatory data for phenomena previously observed or serving to point to means for more adequate utilization of heat for the practical purpose of sterilization.

APPENDIX
PART I

Raw data for calculations of thermal destruction tables and curves

DATA SET I. 0.01M magnesium acetate in 0.01M phosphate buffer pH 7.0.

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 5000000(5.00×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
30 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
40 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
50 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60 sec	55	150	35	102	180	182	135	160	tntc	173
70 sec	13*	60*	15*	81*	82*	50*	40*	87*	64*	47*
80 sec	17	11	06	30	20	02	03	26	06	01
90 sec	00	01	04	00	01	00	00	00	00	00
2 min	00	00	00	00	00	00	00	00	00	00
2½ min	00	00	00	00	00	00	00	00	00	00
3 min	00	00	00	00	00	00	00	00	00	00

Initial Count: 3670000(3.67×10^6)

	Trials					
	1	2	3	4	5	6
Time						
40 sec	tntc	tntc	tntc	tntc	tntc	tntc
50 sec	tntc	tntc	tntc	tntc	tntc	tntc
60 sec	tntc	tntc	tntc	tntc	tntc	tntc
70 sec	175	240	192	121	210	240
80 sec	54*	64*	31*	16*	28*	73*
90 sec	00	15	00	05	00	19
2 min	00	00	00	00	00	00
2½ min	00	00	00	00	00	00
3 min	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET I continued

Temperature:
Recorded: 140 F
Actual: 139.5 F

Initial Count: 3670000(3.67×10^6)

	Trials									
Time	1	2	3	4	5	6	7	8	9	10
1½ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
1 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2 min	250	tntc	tntc	186	268	tntc	tntc	tntc	tntc	tntc
2¼ min	64	88	82	31	76	152	tntc	80	tntc	tntc
2½ min	26*	27*	30*	17*	37*	81*	90*	27*	81*	71*
2 min	16	11	19	12	11	04	16	01	03	05
3 min	10	00	03	10	03	07	09	01	01	01
3¼ min	00	04	03	04	00	03	00	00	00	00
3½ min	00	00	00	00	00	00	00	00	00	00
3 min	00	00	00	00	00	00	00	00	00	00

Initial Count: 4330000(4.33×10^6)

	Trials								
Time	1	2	3	4	5	6	7	8	9
30 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
90 sec	tntc	tntc	tntc	tntc	104	tntc	tntc	tntc	tntc
2 min	84	60*	45*	124*	36*	tntc	96*	164*	tntc
2½ min	23*	06	04	08	00	60*	00	08	62*
3 min	03	00	00	00	00	00	00	00	00
3½ min	00	00	00	00	00	00	00	00	00
4 min	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET I continued

Temperature:
Recorded: 136 F
Actual: 135.4 F

Initial Count: 5330000(5.33×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
3 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
4 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
6 min	210	210	193	190	117	36	150	187	tntc	
7 min	70*	47*	37*	30*	10*	15*	30*	50*	81*	
8 min	17	03	05	08	01	02	04	07	05	
9 min	01	00	00	01	00	00	01	00	00	
10 min	00	00	00	00	00	00	00	00	00	
11 min	00	00	00	00	00	00	00	00	00	
12 min	00	00	00	00	00	00	00	00	00	

Initial Count: 4000000(4.00×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
3 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
4 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
6 min	tntc	198	250	202	63	150	187	tntc	199	123
7 min	76*	20*	28*	57*	38*	55*	46*	87*	34*	40*
8 min	17	01	01	03	01	02	06	26	09	08
9 min	03	00	00	01	00	00	02	04	01	01
10 min	07	00	00	01	00	00	01	01	01	00
11 min	00	00	00	00	00	00	00	00	00	00
12 min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET II. 0.01M citric acid in 0.01M phosphate buffer pH 7.0.

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 5000000(5.00×10^6)

	Trials										
	1	2	3	4	5	6	7	8	9	10	11
Time											
45 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
75 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
90 sec	tntc	300	250	240	182	248	tntc	tntc	260	tntc	tntc
105sec	84*	34*	65*	50*	25*	50*	52*	108*	57*	59*	63*
2 min	05	02	00	00	17	43	00	18	12	04	08
2½ min	00	00	00	00	00	00	00	00	00	00	00
3 min	00	00	00	00	00	00	00	00	00	00	00
3½ min	00	00	00	00	00	00	00	00	00	00	00
4 min	00	00	00	00	00	00	00	00	00	00	00

Temperature:
Recorded: 140 F
Actual: 139.5 F

Initial Count: 3670000(3.67×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
90 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
105sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2 min	214	236	tntc	tntc	237	tntc	tntc	tntc	tntc	tntc
2¼ min	66	38	98	71	113	94	150	62	84	70
2½ min	22*	23*	55*	23*	30*	46*	79*	57*	92	56*
2 min	12	10	48	14	07	48	60	18	52*	70
3 min	03	06	21	01	02	08	29	05	24	05
3¼ min	01	01	15	00	00	05	16	03	12	11
3½ min	03	00	00	01	00	01	02	00	03	00
3 min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET II continued

Initial Count: 4330000(4.33×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
75 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
90 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
105sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2 min	246	150	tntc	173	110	260	280	tntc	182	tntc
2¼ min	153	53	22	50	31	69	tntc	tntc	tntc	tntc
2½ min	138	14*	17*	24*	14*	20*	20*	49*	25*	83*
2 min	14*	03	11	09	17	00	00	00	00	00
3 min	00	00	04	00	01	00	00	00	00	00
3¼ min	00	00	00	00	00	00	00	00	00	00
3½ min	00	00	00	00	00	00	00	00	00	00

Temperature:
Recorded: 136 F
Actual: 135.4 F

Initial Count: 4670000(4.67×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
7½ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
8 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
8½ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
9 min	297	177	199	140	165	282	203	232	200	174
9½ min	187	167	45	120	130	117	136	180	110	100
10 min	71*	51*	41*	66*	47*	66*	54*	87*	69*	78*
10½ min	11	09	22	57	09	40	16	33	27	32
11 min	06	04	06	06	03	09	14	22	14	08
11½ min	03	00	00	02	02	05	10	03	01	03
12 min	00	00	00	01	00	04	07	00	00	00
12½ min	00	00	00	00	00	00	00	00	00	00
13 min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET II continued

Initial Count: 5330000(5.33×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
4	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
6	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
7	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
8	min	85	134	87	180	55	56	28*	39*	35*	
9	min	34*	31*	41*	71*	47*	34*	04	07	05	12*
10	min	05	07	28	31	06	12	01	05	01	01
11	min	01	01	01	04	01	00	00	00	00	00
12	min	00	00	00	00	00	00	00	00	00	00
13	min	00	00	00	00	00	00	00	00	00	00

Initial Count: 3000000(3.00×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
5	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
6	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
7	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
8	min	216	178	240	tntc	98	145	200	287	193	171
9	min	80*	62*	79*	152	34*	45*	65*	97*	61*	40*
10	min	42	35	23	51*	09	10	23	28	16	03
11	min	06	28	12	30	04	03	07	12	11	03
12	min	09	01	01	07	00	02	00	00	02	01
13	min	02	03	02	03	00	00	00	00	00	00
14	min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET II continued

Initial Count: 3000000(3.00×10^6)

	Trials								
	1	2	3	4	5	6	7	8	9
Time									
4 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
6 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
7 min	240	200	287	tntc	tntc	tntc	64	tntc	tntc
8 min	16*	12*	87*	79*	180	150	14*	180	163
9 min	09	05	13	19	73*	28*	00	17*	30*
10 min	01	02	05	10	16	15	00	04	11
11 min	00	00	01	06	06	04	00	02	04
12 min	00	00	00	01	01	00	00	01	01
13 min	00	00	00	00	01	00	00	00	00
14 min	00	00	00	00	00	00	00	00	00
15 min	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET III. 0.85% socium cholride in 0.01M phosphate buffer pH 5.4.

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 6330000(6.33×10^6)

	Trials									
Time	1	2	3	4	5	6	7	8	9	10
40 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
50 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
70 sec	06	210	240	tntc	197	tntc	tntc	tntc	tntc	240
80 sec	20*	16*	16*	150	38	120	250	47*	54*	11*
90 sec	00	05	05	28*	26*	26*	60*	02	02	02
2 min	00	00	00	00	00	00	04	00	00	00
$2\frac{1}{2}$ min	00	00	00	00	00	00	00	00	00	00
3 min	00	00	00	00	00	00	00	00	00	00
$3\frac{1}{2}$ min	00	00	00	00	00	00	00	00	00	00
4 min	00	00	00	00	00	00	00	00	00	00

Initial Count: 5330000(5.33×10^6)

	Trials									
Time	1	2	3	4	5	6	7	8	9	10
50 sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60 sec	tntc	tntc	tntc	tntc	tntc	240	tntc	tntc	tntc	tntc
70 sec	tntc	tntc	tntc	tntc	tntc	91	250	tntc	94	144
80 sec	tntc	210	tntc	tntc	tntc	10*	110	56*	28*	53*
90 sec	56*	45*	180	137	45*	01	45*	03	03	15
105 sec	07	06	50*	56*	08	00	10	00	00	00
2 min	00	03	03	05	05	00	02	00	00	00
$2\frac{1}{2}$ min	00	00	00	00	03	00	00	00	00	00
3 min	00	00	00	00	00	00	00	00	00	00

*Count used in D value calculation.

DATA SET III continued

Temperature:
Recorded: 142 F
Actual: 141.8 F

Initial Count: 4000000(4.00×10^6)

Time		Trials 1	2	3	4	5	6	7	8	9	10
30	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
90	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2	min	tntc	tntc	tntc	240	tntc	tntc	tntc	tntc	250	190
2½	min	138*	50*	36*	14*	46*	102*	130*	240	16*	23*
3	min	01	00	00	00	00	00	02	16*	01	00
3½	min	00	00	00	00	00	00	00	00	00	00
4	min	00	00	00	00	00	00	00	00	00	00
4½	min	00	00	00	00	00	00	00	00	00	00

Temperature:
Recorded: 140 F
Actual: 139.5 F

Initial Count: 5330000(5.33×10^6)

Time		Trials 1	2	3	4	5	6	7	8	9	10
2	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
4	min	302	183	54*	264	76*	105*	237	150	287	100
4½	min	26*	85*	39	70*	05	18	40*	65*	27*	25*
5	min	05	12	05	20	00	00	00	00	00	00
5½	min	03	00	03	07	00	00	00	00	00	00
6	min	00	00	00	06	00	00	00	00	00	00
6½	min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET III continued

Initial Count: 4330000(4.33×10^6)

Time		Trials 1	2	3	4	5	6	7	8	9	10
1½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3½	min	202	183	193	101	102	113	104	161	127	106
4	min	18*	46*	52*	16*	16*	22*	14*	23*	49*	48*
4½	min	08	06	09	10	02	02	01	00	07	04
5	min	06	01	00	04	03	01	00	00	03	01
5½	min	00	00	00	01	00	00	00	00	00	00
6	min	00	00	00	00	00	00	00	00	00	00

Initial Count: 4000000(4.00×10^6)

Time		Trials 1	2	3	4	5	6	7	8	9	10
1	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
1½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3	min	tntc	tntc	tntc	284	tntc	tntc	tntc	tntc	tntc	tntc
3½	min	87	42*	176	78	93	108	168	76	tntc	117
4	min	14*	03	54*	15*	18*	26*	42*	15*	78*	30*
4½	min	01	00	02	01	03	03	03	06	36	04
5	min	00	00	01	00	00	00	00	01	15	00
5½	min	00	00	00	00	01	00	00	00	01	00

* Count used in D value calculation.

DATA SET III continued

Temperature:
Recorded: 136 F
Actual: 135.4 F

Initial Count: 5330000(5.33×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
8	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
9	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
10	min	131	108	110	217	103	110	tntc	tntc	tntc	tntc
11	min	48*	52*	84*	93*	44*	31*	140	220	162	120
12	min	14	25	13	28	27	10	79*	97*	62*	44*
13	min	02	04	08	07	08	03	21	23	04	11
14	min	04	01	00	01	01	00	10	11	00	02
15	min	01	01	00	01	00	00	04	06	00	00
16	min	00	00	00	00	00	00	00	01	00	00
17	min	00	00	00	00	00	00	00	00	00	00

Initial Count: 4000000(4.00×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
8	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
9	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
10	min	tntc	194	86	266	217	180	121	210	82	293
11	min	143	50*	19*	28*	57*	61*	14*	44*	17*	71*
12	min	88	19	06	09	14	27	02	12	04	23
13	min	15	01	02	07	02	13	02	09	01	08
14	min	05	00	01	02	04	02	01	01	00	02
15	min	01	00	00	01	00	00	00	00	00	00
16	min	00	00	00	00	00	00	00	00	00	00
17	min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET IV. 0.85% sodium chloride in 0.01M phosphate buffer pH 7.0.

Temperature:
Recorded: 142 F
Actual: 141.8 F

Initial Count: 4670000(4.67×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
40	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
50	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
70	sec	104	115	236	102	136	164	86	60	187	140
80	sec	29*	36*	45*	28*	29*	37*	25*	22*	26*	29*
90	sec	18	05	32	01	05	06	09	07	01	01
105	sec	03	01	03	01	00	00	00	01	00	00
2	min	02	00	00	00	00	00	00	00	00	00
$2\frac{1}{2}$	min	00	00	00	00	00	00	00	00	00	00
3	min	00	00	00	00	00	00	00	00	00	00

Initial Count: 4000000(4.00×10^6)

		Trials					
		1	2	3	4	5	6
Time							
30	sec	tntc	tntc	tntc	tntc	tntc	tntc
60	sec	tntc	tntc	tntc	tntc	120	42
90	sec	46*	06*	47*	18*	19*	18*
2	min	00	00	00	00	00	00
$2\frac{1}{2}$	min	00	00	00	00	00	00
3	min	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET IV continued

Temperature:
Recorded: 140 F
Actual: 139.5 F

Initial Count: 5000000(5.00×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
1	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
1½	min	239	34*	tntc	96	tntc	tntc	tntc	tntc	tntc	tntc
2	min	41*	01	32*	10*	41*	27*	40*	55*	89*	47*
2½	min	04	02	04	06	05	03	05	07	06	01
3	min	02	00	00	02	01	00	01	03	00	00
3½	min	01	00	00	00	00	00	00	02	00	01
4	min	00	00	00	00	00	00	00	00	00	00
4½	min	00	00	00	00	00	00	00	00	00	00
5	min	00	00	00	00	00	00	00	00	00	00
5½	min	00	00	00	00	00	00	00	00	00	00
6	min	00	00	00	00	00	00	00	00	00	00

Initial Count: 4000000(4.00×10^6)

		Trials										
		1	2	3	4	5	6	7	8	9	10	11
Time												
1	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
1½	min	tntc	tntc	102*	tntc	tntc	tntc	64	66	tntc	tntc	tntc
2	min	80*	26*	00	09*	28*	15*	16*	15*	12*	14*	45*
2½	min	03	02	00	00	06	01	01	10	01	09	02
3	min	01	00	00	00	00	00	00	06	00	00	01
3½	min	00	00	00	00	00	00	00	00	00	00	00
4	min	00	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET IV continued

Temperature:
Recorded: 136 F
Actual: 135.4 F

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
3	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
4	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
6	min	tntc	tntc	tntc	tntc	tntc	tntc	293	tntc	164	264
7	min	107*	219	83*	58*	77*	26*	92*	58*	57*	32*
8	min	08	30*	05	11	14	06	01	08	03	02
9	min	04	02	02	00	01	01	00	03	00	00
10	min	01	00	01	01	00	00	00	00	00	00
11	min	00	01	00	00	00	00	00	00	00	00
12	min	00	00	00	00	00	00	00	00	00	00
13	min	00	00	00	00	00	00	00	00	00	00
14	min	00	00	00	00	00	00	00	00	00	00
15	min	00	00	00	00	00	00	00	00	00	00

Initial Count: 3670000(3.67×10^6)

		Trials				
		1	2	3	4	5
Time						
4	min	tntc	tntc	tntc	tntc	tntc
5	min	tntc	tntc	tntc	tntc	tntc
6	min	172	tntc	250	287	tntc
7	min	32*	92*	48*	49*	95*
8	min	04	11	03	09	14
9	min	01	00	00	00	01
10	min	01	00	00	01	00
11	min	00	00	00	00	00
12	min	00	00	00	00	00
13	min	00	00	00	00	00
14	min	00	00	00	00	00
15	min	00	00	00	00	00

* Count used in D value calculation.

DATA SET V. 8.5% sodium chloride in 0.01M phosphate buffer pH 5.4.

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 4330000(4.33×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
60	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
90	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3½	min	tntc	240	tntc	tntc	tntc	tntc	tntc	tntc	100	tntc
4	min	150	64*	170	83*	240	140	250	84	48*	92*
4½	min	47*	08	27*	09	25*	34*	29*	12*	05	02
5	min	01	00	00	00	00	00	09	00	00	00
5½	min	01	00	00	00	00	00	01	00	00	00
6	min	00	00	00	00	00	00	00	00	00	00
6½	min	00	00	00	00	00	00	00	00	00	00
7	min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculations.

DATA SET V continued

Temperature:
Recorded: 142 F
Actual: 141.8 F

Initial Count: 4000000(4.00×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
3	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
4	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
4½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5½	min	tntc	tntc	192	150	240	tntc	tntc	tntc	tntc	tntc
6	min	240	74*	55*	42*	87	92	tntc	tntc	tntc	tntc
6½	min	97*	26	07	04	19*	26*	182	250	tntc	130
7	min	03	24	00	03	05	07	64*	44*	80*	28*
7½	min	00	00	00	00	02	00	08	24	24	06
8	min	00	00	00	00	00	00	02	05	02	00
8½	min	00	00	00	00	00	00	00	00	00	00
9	min	00	00	00	00	00	00	00	00	00	00
9½	min	00	00	00	00	00	00	00	00	00	00
10	min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET V continued

Temperature:
Recorded: 140 F
Actual: 139.5 F

Initial Count: 4330000(4.33×10^6)

	Trials							
	1	2	3	4	5	6	7	8
Time								
$7\frac{1}{2}$ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
8 min	tntc	tntc	tntc	187	tntc	tntc	tntc	tntc
$8\frac{1}{2}$ min	tntc	tntc	tntc	202	tntc	280	280	280
9 min	tntc	tntc	tntc	52	236	134	276	277
$9\frac{1}{2}$ min	tntc	tntc	tntc	05	79	38	40	55
10 min	260	99	140	15*	54	34*	26*	29*
$10\frac{1}{2}$ min	40*	31*	50*	02	24*	16	12	11
11 min	09	00	10	00	03	02	06	10
12 min	00	00	00	00	00	00	00	00
13 min	00	00	00	00	00	00	00	00

Initial Count: 5330000(5.33×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
$7\frac{1}{2}$ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
8 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
$8\frac{1}{2}$ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
9 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
$9\frac{1}{2}$ min	216	145	249	273	171	75	134	77	100	164
10 min	45*	63*	77*	130*	90*	30*	60*	32*	46*	74*
$10\frac{1}{2}$ min	37	39	10	27	39	00	26	31	00	00
11 min	20	00	00	00	00	00	00	07	00	00
$11\frac{1}{2}$ min	05	00	00	00	00	00	00	00	00	00
12 min	02	00	00	00	00	00	00	00	00	00
$12\frac{1}{2}$ min	01	00	00	00	00	00	00	00	00	00
13 min	01	00	00	00	00	00	00	00	00	00
$13\frac{1}{2}$ min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET VI. 8.5% sodium chloride in 0.01M phosphate buffer pH 7.0.

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 4000000(4.00×10^6)

Time		Trials 1	2	3	4	5	6	7	8	9	10
30	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
60	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
90	sec	tntc	tntc	127*	140*	tntc	tntc	180	tntc	tntc	tntc
2	min	14*	40*	02	01	tntc	tntc	16*	tntc	tntc	tntc
2½	min	00	02	00	01	28*	22*	00	13*	17*	12*
3	min	00	00	00	00	00	00	00	00	00	00
3½	min	00	00	00	00	00	00	00	00	00	00
4	min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET VI continued

Temperature:
Recorded: 142 F
Actual: 141.8 F

Initial Count: 3670000(3.67×10^6)

		Trials					
		1	2	3	4	5	6
Time							
90	sec	tntc	tntc	tntc	tntc	tntc	tntc
2	min	tntc	tntc	tntc	tntc	tntc	tntc
2½	min	260	257	240	tntc	150	280
3	min	54*	30*	37*	94*	34*	40*
3½	min	03	04	01	02	01	03
4	min	01	01	01	01	00	01
4½	min	00	00	00	00	00	00
5	min	00	00	00	00	00	00
5½	min	00	00	00	00	00	00

		7	8	9	10	11	12
Time							
90	sec	tntc	tntc	tntc	tntc	tntc	tntc
2	min	tntc	tntc	tntc	tntc	tntc	tntc
2½	min	tntc	tntc	tntc	tntc	tntc	tntc
3	min	120*	88*	136	tntc	82*	76*
3½	min	10	12	22*	139	00	00
4	min	01	02	02	24*	00	00
4½	min	00	00	00	04	00	00
5	min	00	00	00	00	00	00
5½	min	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET VI continued

Temperature:
Recorded: 140 F
Actual: 139.5 F

Initial Count: 5330000(5.33×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
4	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
4½	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
5	min	160	227	tntc	tntc	131	59	119	138	104	80
5½	min	44*	39*	74*	84*	33*	33*	23*	54*	13*	17*
6	min	00	00	33	24	12	16	13	12	02	01
6½	min	00	00	00	00	00	02	01	01	00	01
7	min	00	00	00	00	00	00	00	00	00	00
7½	min	00	00	00	00	00	00	00	00	00	00
8	min	00	00	00	00	00	00	00	00	00	00
8½	min	00	00	00	00	00	00	00	00	00	00
9	min	00	00	00	00	00	00	00	00	00	00
9½	min	00	00	00	00	00	00	00	00	00	00
10	min	00	00	00	00	00	00	00	00	00	00
10½	min	00	00	00	00	00	00	00	00	00	00
11	min	00	00	00	00	00	00	00	00	00	00
11½	min	00	00	00	00	00	00	00	00	00	00
12	min	00	00	00	00	00	00	00	00	00	00
12½	min	00	00	00	00	00	00	00	00	00	00
13	min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET VII. 39.6% glycerol in 0.01M phosphate buffer pH 7.0.

Temperature:
Recorded: 148 F
Actual: 147.6 F

Initial Count: 1400000(1.4×10^6)

	Trials							
	1	2	3	4	5	6	7	8
Time								
1 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2 min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3 min	06*	26*	16*	120*	144*	30*	35*	58*
4 min	00	00	00	00	03	00	00	00

Temperature:
Recorded: 146 F
Actual: 145.7 F

Initial Count: 1400000(1.4×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
3 min	tntc	tntc	tntc	tntc	353*	tntc	tntc	tntc	tntc	tntc
4 min	56*	tntc	10*	56*	05	28*	55*	50*	84*	85*
5 min	00	20*	00	03	00	00	03	04	09	20
6 min	00	00	00	02	00	00	00	00	00	00
7 min	00	00	00	00	00	00	00	00	00	00

* Count used in D value calculation.

DATA SET VII continued

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 1500000(1.5×10^6)

	Trials					
	1	2	3	4	5	6
Time						
8 min	tntc	tntc	tntc	tntc	tntc	tntc
9 min	tntc	tntc	tntc	tntc	tntc	tntc
10 min	65*	60*	80*	tntc	58*	130*
11 min	02	13	07	33*	21	05
12 min	01	00	00	12	07	01

* Count used in D value calculation.

DATA SET VIII. 15.4 % glycerol in 0.01M phosphate buffer pH 7.0.

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 4300000(4.3×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
$1\frac{1}{2}$ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2 min	tntc	240	tntc	tntc	190	tntc	174	180	tntc	234
$2\frac{1}{2}$ min	20*	34*	37*	58*	20*	51*	29*	50*	53*	41*
3 min	03	08	12	02	03	06	01	01	06	03
$3\frac{1}{2}$ min	01	03	02	00	00	00	00	00	00	00
4 min	00	00	00	00	00	00	00	00	00	00

Temperature:
Recorded: 142 F
Actual: 141.8 F

Initial Count: 4000000(4×10^6)

	Trials									
	1	2	3	4	5	6	7	8	9	10
Time										
$2\frac{1}{2}$ min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
3 min	tntc	tntc	tntc	tntc	tntc	170	tntc	tntc	tntc	tntc
$3\frac{1}{2}$ min	66*	52*	30*	46*	93*	36*	90*	46*	154*	64*
4 min	35	17	06	13	22	10	28	14	60	21
$4\frac{1}{2}$ min	02	12	03	05	09	02	02	02	09	01
5 min	00	02	00	00	02	00	00	00	07	00
$5\frac{1}{2}$ min	00	02	00	00	00	00	00	00	01	00

* Count used in D value calculation.

DATA SET VIII continued

Temperature:
Recorded: 140 F
Actual: 139.5 F

Initial Count: 3000000(3×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
4	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
$4\frac{1}{2}$	min	135	137	144	158	106	133	153	106	87	101
5	min	17*	19*	54*	60*	89*	88*	37*	19*	21*	50*
$5\frac{1}{2}$	min	16	03	23	17	48	47	12	03	03	08
6	min	02	00	07	06	13	13	00	04	01	00
$6\frac{1}{2}$	min	00	00	06	03	02	03	01	01	01	03

* Count used in D value calculation.

DATA SET IX. 0.01M phosphate buffer pH 7.0.

Temperature:
Recorded: 144 F
Actual: 143.7 F

Initial Count: 1200000(1.2×10^6)

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
30	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
1	min	tntc	tntc	172	160	180	tntc	tntc	tntc	tntc	tntc
$1\frac{1}{2}$	min	180*	37*	75*	10*	80*	96*	148*	156*	tntc	40
2	min	00	00	00	00	00	13	03	11	15*	12*

Temperature:
Recorded: 142 F
Actual: 141.8 F

Initial Count: 1900000(1.9×10^6) trials 1-5
1700000(1.7×10^6) trials 6-10

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
30	sec	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
1	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
$1\frac{1}{2}$	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
2	min	53*	180*	36*	104*	tntc	73*	tntc	23*	33*	12*
$2\frac{1}{2}$	min	13	26	04	12	75*	13	120*	01	01	01
3	min	08	01	00	03	10	03	32	00	00	00
$3\frac{1}{2}$	min	00	00	00	00	00	00	03	00	00	00

* Count used in D value calculation.

DATA SET IX continued

Temperature:
Recorded: 136 F
Actual: 135.4 F

Initial Count: 1500000(1.5×10^6) trials 1-7
1900000(1.9×10^6) trials 7-10

		Trials									
		1	2	3	4	5	6	7	8	9	10
Time											
5	min	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc	tntc
6	min	132	tntc	tntc	tntc	220	216	tntc	tntc	tntc	tntc
7	min	140	160	96	54	72	96	120	100	120	52
8	min	24*	45*	32*	19*	26*	47*	64*	45*	30*	23*
9	min	10	10	19	12	10	24	33	16	11	13
10	min	10	02	08	00	02	09	06	13	00	07
11	min	03	01	04	00	02	00	03	06	00	00
12	min	00	02	01	00	00	02	01	00	00	00

* Count used in D value calculation.

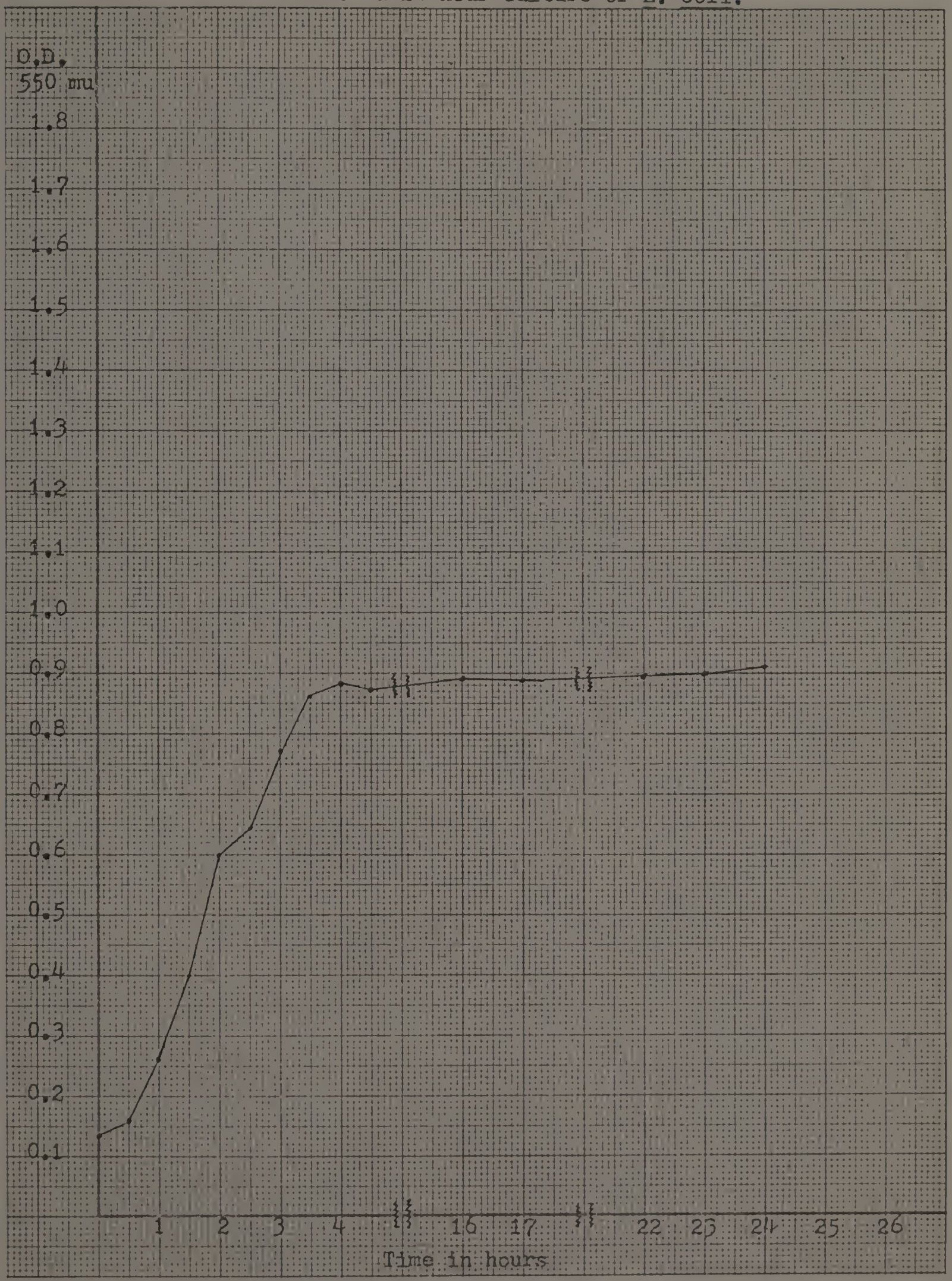
APPENDIX
PART II

Growth curve data for a 24 hour culture of *E. coli*

DATA SET X. Growth curve data for the test organism E. coli ATCC # 11775.

	Optical Density at 550 mu		
Time in hours	Sample 1	Sample 2	Average
0.0	0.1367	0.1249	0.1308
0.5	0.1580	0.1580	0.1580
1.0	0.2518	0.2716	0.2617
1.5	0.382	0.403	0.393
2.0	0.602	0.602	0.602
2.5	0.638	0.648	0.643
3.0	0.757	0.782	0.770
3.5	0.870	0.854	0.862
4.0	0.886	0.870	0.888
4.5	0.870	0.870	0.870
16.0	0.921	0.870	0.896
17.0	0.886	0.886	0.886
22.0	0.890	0.895	0.893
23.0	0.910	0.890	0.900
24.0	0.921	0.903	0.912

FIGURE 10. Growth curve of a 24 hour culture of E. coli.



APPENDIX
PART III

Optical Density reading for the menstra tested at room temperature

DATA SET XI. Optical density readings for 0.01 M magnesium acetate in 0.01 M phosphate buffer pH 7.0 at room temperature.

OD mu	30 sec	3.5 min	10 min	20 min	45 min
230	.584	.691	.647	.590	.600
235	.284	.296	.289	.234	.285
240	.157	.169	.177	.123	.220
245	.134	.131	.147	.197	.183
250	.131	.122	.142	.149	.158
255	.140	.127	.144	.156	.157
260	.146	.136	.152	.160	.162
265	.154	.154	.157	.157	.153
270	.160	.165	.154	.166	.166
275	.160	.170	.160	.156	.165
280	.145	.160	.143	.142	.145
285	.117	.129	.119	.088	.119
290	.089	.093	.088	.061	.071

DATA SET XII. Optical density readings for 0.01 M magnesium acetate in 0.01 M phosphate buffer pH 7.0 at room temperature.

OD mu	30 sec	3.5 min	10 min	20 min	40 min	60 min
230	.062	.055	.066	.114	.081	.053
240	.033	.034	.053	.074	.053	.030
250	.032	.033	.042	.071	.051	.027
255	.032	.031	.038	.069	.047	.026
260	.032	.029	.036	.067	.042	.030
265	.031	.030	.036	.063	.037	.030
270	.030	.028	.035	.063	.038	.027
275	.027	.024	.035	.060	.030	.026
280	.024	.018	.032	.050	.024	.025
290	.020	.016	.027	.049	.017	.015

DATA SET XIII. Optical density readings for 0.01 M magnesium acetate in 0.01 M phosphate buffer pH 7.0 at room temperature.

OD mu	30 sec	10 min	20 min	48 min
230	.960	.730	.790	.514
235	.500	.590	.389	.252
240	.270	.328	.191	.132
250	.172	.225	.117	.087
255	.177	.240	.126	.090
260	.200	.230	.248	.239
265	.230	.318	.173	.110
270	.265	.360	.200	.112
280	.268	.375	.204	.104
285	.167	.200	.125	.073
290	.110	.167	.074	.056

DATA SET XIV. Optical density readings for 0.85% sodium chloride in 0.01 M phosphate buffer pH 7.0 at room temperature.

OD mu	30 sec	2 min	10 min	20 min	45 min
230	.705	.670	.630	.678	.669
235	.374	.354	.369	.379	.371
240	.238	.239	.232	.239	.239
245	.190	.188	.185	.193	.193
250	.176	.176	.173	.178	.179
255	.176	.175	.173	.179	.178
260	.184	.184	.180	.181	.183
265	.191	.189	.185	.189	.189
270	.198	.192	.186	.192	.191
275	.198	.190	.185	.191	.188
280	.188	.182	.175	.182	.179
285	.162	.156	.151	.156	.154
290	.136	.129	.120	.131	.125

DATA SET XV. Optical density readings for 0.85% sodium chloride pH 5.4 at room temperature.

OD mu	30 sec	4 min	10 min	20 min	45 min
230	.660	.740	.670	.670	.670
235	.391	.422	.391	.390	.395
240	.254	.278	.261	.259	.252
245	.210	.228	.212	.209	.210
250	.193	.212	.201	.199	.199
255	.193	.208	.199	.207	.202
260	.199	.212	.204	.208	.205
265	.205	.221	.212	.212	.213
270	.208	.222	.214	.213	.214
275	.211	.222	.214	.209	.210
280	.201	.218	.203	.202	.201
285	.179	.195	.179	.177	.176
290	.143	.164	.146	.144	.145

DATA SET XVI. Optical density readings for 8.5% sodium chloride in 0.01 M phosphate buffer pH 7.0 at room temperature.

OD mu	30 sec	5 min	10 min	45 min
230	.720	.720	.720	.720
235	.417	.490	.420	.419
240	.289	.292	.288	.280
245	.238	.238	.239	.235
250	.225	.236	.225	.222
255	.225	.236	.225	.222
260	.230	.251	.230	.229
265	.238	.251	.234	.232
270	.237	.251	.238	.235
275	.236	.247	.255	.232
280	.229	.230	.229	.228
285	.202	.208	.204	.207
290	.176	.178	.176	.175

DATA SET XVII. Optical density readings for 8.5% sodium chloride pH 5.4 at room temperature.

OD mu	30 sec	10 min	15 min	20 min	45 min
230	.645	.680	.720	.725	.700
235	.365	.451	.418	.419	.381
240	.200	.269	.280	.288	.260
245	.208	.225	.236	.237	.219
250	.199	.215	.222	.228	.205
255	.198	.212	.221	.224	.206
260	.202	.219	.227	.230	.210
265	.209	.221	.234	.236	.221
270	.209	.222	.236	.237	.221
275	.209	.221	.237	.236	.219
280	.200	.214	.229	.230	.212
285	.179	.189	.207	.220	.190
290	.151	.164	.178	.201	.162

DATA SET XVIII. Optical density readings for 15.4% glycerol in 0.01 M phosphate buffer pH 7.0 at room temperature.

OD mu	30 sec	2 min	10 min	20 min	40 min	60 min
230	.710	.720	.715	.735	.730	.660
235	.381	.370	.372	.395	.400	.350
240	.247	.250	.252	.279	.289	.224
245	.249	.253	.277	.259	.274	.264
250	.251	.250	.246	.280	.301	.292
255	.240	.247	.283	.316	.338	.315
260	.247	.256	.294	.329	.346	.330
265	.253	.269	.292	.320	.351	.330
270	.261	.273	.296	.326	.330	.307
275	.247	.251	.265	.282	.294	.272
280	.208	.220	.230	.234	.246	.225
285	.165	.171	.174	.181	.181	.160
290	.110	.109	.125	.125	.125	.105

APPENDIX
PART IV

Standard curves used in the determination of protein and ribose from heated cells

FIGURE 11. Standard curve for D-Ribose determination by Orcinol Reagent.

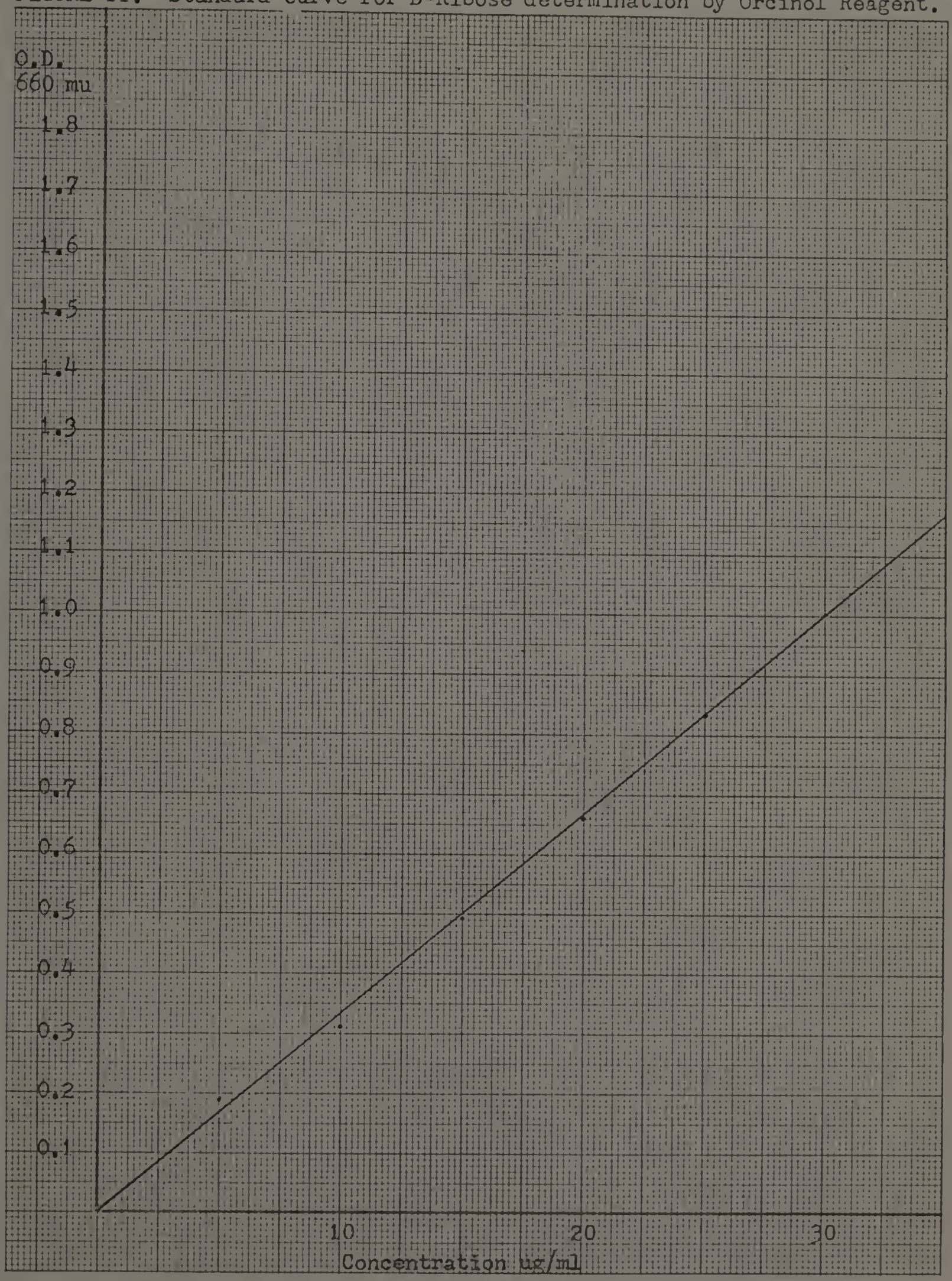
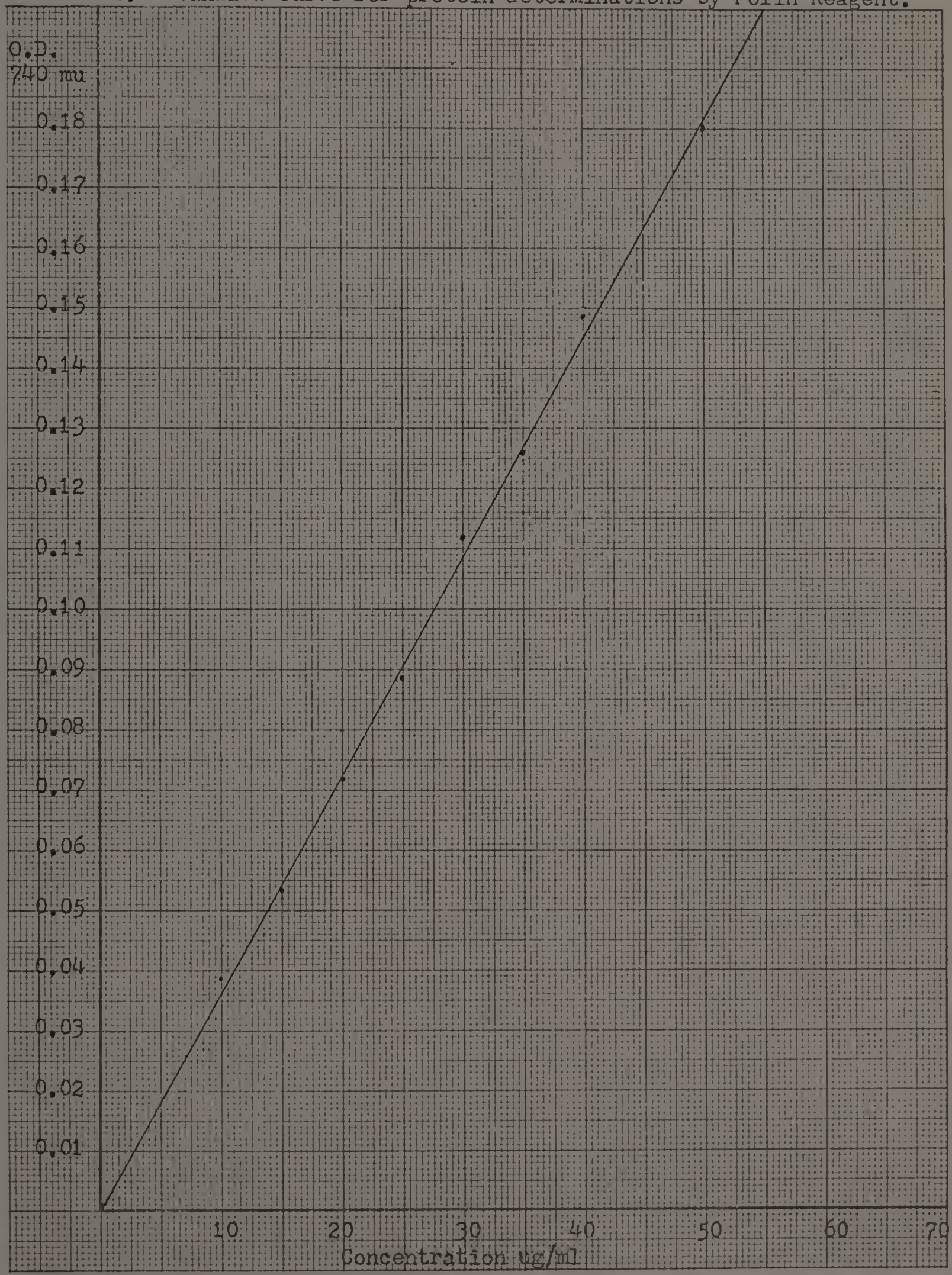


FIGURE 12. Standard curve for protein determinations by Folin Reagent.



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