GROWTH SUPPORT POTENTIAL OF IRRADIATED FOODS FOR MICROORGANISMS OF PUBLIC HEALTH SIGNIFICANCE

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B.S. University of Massachusetts
(1952)

SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

May 14, 1960

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Dear Sir:

Submitted herewith is a thesis titled, "Growth Support Potential of Irradiated Foods for Microorganisms of Public Health Significance", in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Food Technology.

Respectfully submitted,

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ABSTRACT

GROWTH SUPPORT POTENTIAL OF IRRADIATED FOODS FOR MICROORGANISMS OF PUBLIC HEALTH SIGNIFICANCE

by

MYRON SOLBERG

Submitted to the Department of Food Technology on May 14, 1960 in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Methods for the measurement of growth response of microorganisms on the surface of solid substrates were developed and evaluated.

The growth response of Staphylococcus aureus was observed on nutritionally comparable solid and liquid media. Differences in growth response which may have been attributable to the physical state of the medium were observed during the adjustment, logarithmic, and stationary phases of the growth cycle of the organism.

The growth response of Staph. aureus and Escherichia coli during the adjustment, logarithmic, and stationary phases of the growth cycle was observed on raw, heat processed, radiation pasteurized, and radiation sterilized chicken meat.

A growth inhibiting effect was observed on radiation processed chicken meat during the adjustment phase of both microorganisms studied. The duration of the adjustment phase was increased as the total dose of ionizing radiations applied to the chicken meat was increased, regardless of the rate at which the radiations were applied.

Both heat processed and radiation processed chicken showed a greater growth support potential for E. coli during the logarithmic phase of the growth than did raw chicken meat. In the case of the Staph. aureus the processed chicken meats were less effective in supporting growth during the logarithmic growth phase. This was indicated by the increased time required for reproduction of the organism on all of the processed chicken samples with the exception of the initial response on the heat processed chicken samples.

The growth response of Staph. aureus and E. coli on chicken meat during the stationary phase of the growth cycle was, in general, related to the growth rates observed during the logarithmic phase of growth on chicken which had been subjected to similar treatment. The greater the growth rate during the logarithmic phase, the greater the final concentration of cells during the stationary phase.

Evidence was presented which indicated that the growth rate of E. coli on radiation processed chicken and on heat processed chicken were the same. The growth rate of E. coli on both the heat processed and the radiation processed chicken

meat was slightly greater than that on the raw chicken meat. Under these circumstances the use of coliform concentrations as an index of sanitary quality would be just as valid for radiation processed foods as it is for raw or heat processed foods.

It was also shown that longer periods of time would be required to obtain a dangerous (toxic) concentration of Staph. aureus on radiation processed chicken meat than would be required on raw chicken meat assuming inoculation with the same concentration of organisms. Thus, radiation processing may provide food products which are somewhat less likely to transmit food poisoning of the type caused by Staph. aureus.

Thesis Supervisor: Professor John T.R. Nickerson
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ACKNOWLEDGEMENTS

The author is grateful to the late Professor Bernard E. Proctor for his guidance and inspiration during the initial phases of this thesis. He also wishes to express his gratitude to Professor John T. R. Nickerson who served as thesis advisor during the later phases of the studies and offered his advice and time throughout the conduct of the investigations and the preparation of this manuscript.

Thanks are also due to Professor Samuel A. Goldblith for his encouragement and guidance of the author during the course of his study at the Massachusetts Institute of Technology.

Appreciation is expressed to Dr. C. W. Fifield and Dr. Gerald Silverman for the countless hours which each devoted to the author in discussion and interpretation of the results obtained during the investigations.

To the many other members, both past and present, of the Department of Food Technology, who have offered their aid, advice, and materials, the author is sincerely thankful. Special mention should be made of Mr. Henry Bartlett, Mrs. Robert Whelan, and Miss Marion Nickerson in appreciation of the technical assistance which was offered by these persons during various phases of the study.

The gratitude of the author is also expressed to the National Institutes of Health without the grant of which this work could not have been carried out.

For their continual encouragement and faith, the author is indebted to his parents, Mr. and Mrs. Alexander Solberg and to his parents through marriage, Mr. and Mrs. Samuel Bernstein.

Last, but indeed most of all, the author expresses his deep felt gratitude to his wife, Rona, for her love, patience, faith, encouragement, and self sacrifice through the years.

I. INTRODUCTION

The need for man to preserve food and man's search for a more efficient and a more satisfactory means to preserve food have persisted through the ages. Archeologists have found evidence of food preservation in the earliest civilizations. Prehistoric man probably relied on nature for food preservation. Drying by exposure to the sun and dry storage of certain low moisture foods, such as nuts and cereal grains, were probably the earliest methods of food preservation.

Earliest recorded history tells of widely practiced food preservation. The origins, in time, of salting, smoking and fermentation as methods of food preservation are unknown but they appear to have been used concurrently in widely separated civilizations. The use of snow by the early Romans was one of the first reports of refrigeration in conjunction with food products, though it is believed to have been used by the Chinese long before the era of recorded history.

In general, there was little progress in the art of food preservation through the centuries until the development of canning by Nicholas Appert in 1810. The application of true science did not occur until after the turn of the century when the science of bacteriology was first applied to the canning industry. During the 20th century scientific methods have been employed in the development of several techniques of food preservation. Among these are canning, freezing and artificial drying. The high state to which these food

preservation techniques have developed is, in fact, the very basis of modern civilization with its large urban centers. However, the search for new and better methods for increasing the storage life of foods continues and has led, in recent years, to the proposed utilization of ionizing radiations for the preservation of food products. Though no radiation processed food product is being marketed at this time, it is possible that within the next few years foods which have been treated with ionizing radiations will become available in both military rations and in ordinary commerce.

The development of a food process by ionizing radiation has been approached on a much more scientific basis than any previously developed food preservation technique. The answers to many questions pertaining to toxicology, nutrition, chemistry, and bacteriology are being sought prior to commercial introduction of the process. Much of the information sought is at the fundamental rather than the applied level, in the hope that the answers obtained may be utilized on a broad basis and not be restricted to a specific food product under a specific group of conditions.

Though much attention has been given to the radioresistance of microorganisms, radiation induced off-flavors, chemical changes due to radiation treatment, and radiation effect upon nutritional value, little or no attention has been focused upon the effect of ionizing radiations upon the product from the standpoint of its suitability for the growth of microorganisms of public health significance. This study, to

determine the effect of ionizing radiations upon chicken meat as a substrate for the growth of food poisoning and related microorganisms, was undertaken to fill this void which seems to exist in the overall program for the radiation preservation of food.

This study includes the growth characteristics of the microorganisms involved, not only on radiation pasteurized and radiation sterilized food, but also on the raw and thermally processed food prepared in accordance with commercial methods.

This thesis was also designed to obtain fundamental information relating to growth phenomena of microorganisms on solid media. This includes growth rates over periods of many generations, growth inception periods, and growth in the period after a maximum or near maximum number of viable cells has been attained. Comparatively little information pertaining to growth of microorganisms on solid media can be found in scientific literature due to the difficulties encountered in measurement of the growth under these conditions.

The microorganisms chosen for the investigations were: Escherichia coli, because it is utilized as a common indicator of fecal contamination and thus is of great public health significance; and Staphylcoccus aureus, because of the fact that it is the chief cause of food poisoning in the United States of America.

The information obtained from these studies may help determine the adequacy or inadequacy of today's food handling

techniques when they are applied to radiation processed foods. The studies may, therefore, help to determine whether a need exists for the development of new food handling techniques for use in conjunction with radiation processing. The more basic information gained concerning microbial growth on solid media will add to the growing fund of knowledge pertaining to microorganisms and may aid in the solution of problems encountered by future investigators.

In summary, the objectives of this thesis are threefold:

1. To determine whether radiation treated food is potentially more dangerous than foods handled by ordinary methods with respect to the growth of food poisoning bacteria.

2. To derive information concerning the behavior of microorganisms when grown in a radiation treated nutrient material.

3. To compare the use of coliform concentrations as an index of sanitary quality in radiation processed foods and commercially handled foods.

II. LITERATURE

A. Stability of microorganisms in dilution blanks

The problems associated with the survival of microorganisms in dilution blanks are not new. Only a limited amount of attention has been given to this subject in the past since standard sampling methods have been designed in such a manner that holding time in dilution blanks is kept to a minimum. The fact that different dilution blanks are recommended in various procedures of the Recommended Methods for the Microbiological Examination of Foods (1958) offers evidence of the existence of the survival problem, even when foods or other materials are examined by standard methods.

Lamanna and Mallette (1953) summarized the literature pertaining to the survival of washed bacteria and concluded that washed cells continue respiring endogenously when held in a nonnutrient medium and exhibit steadily decreasing plate counts during the starvation period.

Winslow and Falk (1923) studied the effect of the pH of distilled water and physiological saline at 37°C upon the survival of E. coli. They reported total survival at pH 6.0 in distilled water and at pH 6.5 in saline. On both sides of these pH values they observed a rapid loss of viability.

Winslow and Brooke (1927) compared washed and unwashed cells of Bacillus cereus, Bacillus megatherium, Erythrobacillus prodigiosus and E. coli with respect to their ability to survive in dilution blanks. With the exception of E. coli they observed

a rapid loss of cell viability in washed cultures suspended in distilled water. When unwashed cells were subjected to the same treatment they exhibited a considerable degree of survival without exception. In carrying the experiments further, these investigators found that one part of broth in 100 parts of water offered complete protection to the cells. Similar results were obtained with both meat extract and peptone but neither saline nor sugar at isotonic concentrations offered any protection.

While sampling natural water supplies, Butterfield (1932) found that distilled water dilution blanks always resulted in reduced counts, whereas phosphate buffered distilled water yielded a high per cent of survival. He concluded that the hydrogen ion concentration and the mineral salt content of the waters were the most important factors to be considered.

Straka and Stokes (1957) investigated the use of distilled water, physiologic saline, phosphate buffered water, and peptone water containing from 0.5 to 0.001% peptone, as diluents for use in the microbiological examination of food products. They noted a rapid decrease in viable count during 20 minutes in all of the diluents with the exception of the peptone water. They concluded that since 0.01% peptone water was only effective in some of the experiments, while 0.1% peptone water was effective in every experiment, 0.1% peptone water should be used as the diluent for the microbiological investigation of food products. One restriction which these authors pointed out was that all dilutions and plating must be completed within one hour because of a multiplication effect which was observed between one and two hours of holding time prior to culturing.

The use of chilled dilution waters and the chilling of cultures to maintain status quo conditions during storage, or during various handling procedures such as grinding or shaking, led to studies of the effect of sudden chilling on microorganisms. Hegarty and Weeks (1940) and more recently Meynell (1958) reported that young cells of E. coli and cultures in the log phase of growth were very susceptible to cold shock, while mature cells or cultures in the stationary phase of growth were not affected by either cold shock or cold storage.

B. The sterility of raw chicken muscle

Tanner (1944) reviewed the literature pertaining to the microbial content of blood and tissues of healthy animals. The presence or absence of microorganisms in live animal tissue is a controversial matter in view of the reports cited. The organisms found in the tissues of healthy animals (beef) were of no pathological significance and did not appear to multiply during cold storage. Another report cited by Tanner showed that when animals were slaughtered under hygienic conditions a low bacterial contamination resulted. The organisms present on the surface of the meat generally exhibited a lag period of some 40 hours at 37°C after which they were capable of rapid multiplication. In studies referring to fowl there were reports cited of both penetration and non-penetration of microorganisms into the flesh, as well as reports of sterile and non-sterile muscle of live animals of this type.

Zender et al. (1958a) reported that in-vivo animal muscle was sterile. The animals studied by these investigators included

lambs, pigs, and rabbits. A report describing a technique by which sterile muscle of lambs and rabbits had been excised was made by Zender et al. (1958b). The method consisted basically of the employment of surgical techniques in an atmosphere of sterile air.

C. Food products as bacteriological substrates

In order to study the growth of microorganisms upon the surface of food products it was desirable to obtain control over the surface area exposed to the microorganisms and the moisture content of the substrate. Scott (1953) utilized food slurries to study the effect of water activity upon S. aureus. Christian and Scott (1953) extended the previous study to Salmonellae. These two studies presented evidence that the duration of the lag, the rate of growth and the total number of cells grown were functions of the water activity of the substrate. The maximum rate of growth for S. aureus in Brain Heart Infusion was found to be approximately 1.9 divisions per hour and the maximum cell concentration reached was 5×10^{10}, however, both of these maxima occurred at different water activity levels. At the water activity which yielded maximum growth rate the maximum cell concentration obtained was only 1×10^{10}. In the case of Salmonella estimates of the lag period were too variable for valid conclusions to be drawn, though all of the results generally confirmed those found for S. aureus. In the Salmonella study, they found that growth on food products occurred at a slightly lower water activity than did growth in broth. Evidence was also presented to show that coliform organisms were comparable with Salmonellae in this respect.

The conversion of the food slurry into a solid substrate would yield a product much closer in nature to a true food and would allow control of water content and surface area. The use of agar as the solidifying agent for the food slurry seemed suitable since Frobisher (1944) described agar as a bacteriologically inert substance. However, Ryan (1952) reported the formation of microcolonies of E. coli on agar in the absence of any added carbon source. Fraser (1953) reported a growth stimulating effect of unpurified agar when it was used in a synthetic medium for the propagation of the common mushroom. J.J. Miller (1959) reported that Versene washed agar supported much less growth of Saccharomyces cerevisiae than did commercial agar. Agar which was purified by washing in pyridine, distilled water and ethanol in accordance with the methods described by Ryan, Beadle and Tatum (1943) has biotin, thiamine, pyridoxine and hypoxanthine as well as some inhibitory substances removed from it.

In order to circumvent the difficulty involved in differentiating between food particles and bacterial colonies, techniques such as the surface swab and the serial dilution (most probable number, decimal dilution) have been used in the past. Angelloti et al. (1958) evaluated the cotton swab technique with respect to the recovery of a known contamination and the precision between successive recoveries of this known contamination. They concluded that the cotton swab technique was low in recovery and low in precision.

Ziegler and Halvorson (1935) found that the serial dilution method was less accurate and less precise than the standard

plate count even when ten tubes were used at each dilution.

The ability of bacteria to reduce 2,3,5 triphenyltetrazolium chloride to the insoluble red formazan form was first reported by Kuhn and Jerchel (1941). Goetz and Tsuneishi (1951) reported that 2,3,5 triphenyltetrazolium chloride (TTC) as a component of bacterial media facilitated the detection and counting of microorganisms. However, Fifield and Hoff (1957) and Weinberg (1953) reported that TTC was a growth inhibitor of many organisms in concentrations as low as .04 and .05%, and could therefore not be utilized to obtain total counts of mixed cultures if the indicator were incorporated into the growth medium.

D. Growth of Microorganisms

The techniques employed in the measurement of growth of microorganisms have been reviewed by Porter (1946), Monod (1949), Lamanna and Mallette (1953), and Oginsky and Umbreit (1954). All of these reviewers presented essentially the same techniques. The methods discussed by Oginsky and Umbreit (1954) included: weight of the culture, volume of cells, protein nitrogen, light scattering, and metabolic activity. Of these only metabolic activity could be employed when a solid substrate was considered.

Bayne-Jones and Rhees (1929) measured the heat produced by growing bacterial cultures. They found that calorimetric techniques did not correlate well with the multiplication rate during the exponential phase of growth.

Hershey and Bronfenbrenner (1938) measured the uptake of oxygen by a growing culture of microorganisms. Their results showed a very large oxygen uptake during the very early stages of

growth followed by a decreased and continually changing uptake during the major portion of the exponential phase of growth.

Monod (1949), after discussing the various techniques for the measurement of bacterial growth, arrived at the following conclusion:

> "Viable counts retain the undisputed privilege of being by far the most sensitive method and of alone permitting differential counting in the analysis of complex populations. In the latter case, relative numbers are generally the significant variable and whether or not the counts give a reasonably accurate estimation of the absolute cell concentrations is unimportant."

The nomenclature for the seven stages of the growth curve for microorganisms, which was originally reported by Buchanan was presented by Porter (1946). Porter (1946) suggested the combination of Buchanan's initial stationary phase and lag phase into a single phase known as "the phase of adjustment". This was the period extending from the initial introduction of inoculum into a medium to the time when the organisms have begun to reproduce at a constant rate.

Mudge and Smith (1939), Porter (1946), Lamanna and Mallette (1953), and more recently Lichstein (1959) reviewed the theories of the lag phase and the factors influencing the initiation of microbial growth. All of the investigations pertaining to the phase of adjustment have not yielded an explanation which does not meet with exceptions.

The logarithmic or exponential phase of growth was reviewed by Gunsales (1951), Lamanna and Mallette (1953), Oginsky and Umbreit (1954), and Wilson and Miles (1955). All of these reviewers showed the growth rate to be a function of the com-

pleteness of the medium in relation to the nutritional requirements of the organism being considered.

Many theories have been developed to explain the stationary phase phenomenon demonstrated by microorganisms. Lamanna and Mallette (1953) reviewed the theories and presented evidence which tended to invalidate each one.

There is little scientific literature pertaining to the growth of microorganisms on solid media. Lamanna and Mallette (1953) present no evidence but hypothesize that there is no fundamental difference between bacterial growth in liquid and on solid media except that a greater population density of organisms per unit volume of environment is attained on a solid medium. These authors offered three reasons for this: first, oxygen availability does not become limiting on solid media as soon as it does in unaerated liquid media; second, toxic metabolic product concentration is reduced in the immediate vicinity of the growing cells due to free diffusion into unoccupied areas of the solid medium; and third, solid media provide a reservoir of fresh nutrients which may diffuse into the areas of bacterial development.

The nutritional adequacy of media may be studied through differences in length of the lag phase, in the rate of growth during the log phase, and in the total population at the stationary phase according to Oginsky and Umbreit (1954).

E. Radiation processing

The advantages of radiation processing and the role which it will play in world affairs have been reviewed by Bailey et al. (1957) in a thorough review of the radiation preservation of food. One of the foods which has been found among the more promising for successful processing with ionizing radiations is chicken. This has been substantiated by the recent announcement in Chemical and Engineering News (1960) that the U.S. Army Quartermaster Corp was planning to continue work toward the successful radiation sterilization of chicken.

Early work by Proctor et al. (1954) and Proctor et al. (1956) showed that sterilization of chicken could be accomplished with doses of 2×10^6 rep of ionizing radiations. Brownell (1957) found that one million rep destroyed all but spore-forming microorganisms in foods. Kempe (1956) inoculated meat with 40,000 spores per gram of Clostridium botulinum and found that 3.7×10^6 rad were required for complete destruction of the organisms. Anderson et al. (1956) reported the isolation of a radio-resistant micrococcus from meat which was as resistant as any of the previously mentioned spore forming microorganisms. Niven (1958) reviewed the microbiological aspects of radiation preservation of foods and cited a study in which it was determined that 4.5×10^6 rad of ionizing radiations would be required, as a minimum, to give the same safety as that which is employed by the canning industry.

Brownell and Purohit (1955) reported on the radio-pasteurization of chicken meat exposed to 1×10^6 rep and stored

at room temperature. They found the product satisfactory after one month though it was slightly rancid and had a slight radiation off-flavor. McGill et al. (1959) reported on low dose pasteurization of chicken. These investigators exposed the chicken to doses of $1x10^5$ rep to $5x10^5$ rep and found the storage temperature after exposure was an important factor in the spoilage by rancidification. Low temperature increased the rate of rancidification while increased temperature increased the rate of spoilage from microbial deterioration.

Proctor et al. (1954) reported that there was less tendency toward off-flavor production in freshly killed chickens subjected to $2-3x10^6$ rep of ionizing radiations than in chickens which were killed some time prior to exposure. These same investigators reported that there was no change in the pH of chicken muscle when it was exposed to 2 million rep.

Blood (1958) carried out a long term feeding study of irradiated chicken to dogs. After 60 weeks there were no differences between the controls and those which were fed the irradiated diet. Richardson (1958) and Phillips (1957) carried out long term rat feeding studies with irradiated chicken. After three generations of rats Richardson reported no differences between controls and the rats on the irradiated diets. After 80 weeks, Phillips found no significant differences between the rats which were fed irradiated chicken and those which were fed control diets.

The sources of ionizing radiations were reviewed by Pomerantz et al. (1957). The high dose rate feature of the

electron accelerator makes its use desirable in food processing. Its low penetrating capability, due to the charged particle which it delivers limits its usefulness to thin samples packaged in film materials. Goldblith and Proctor (1954) described the non-uniform absorption of energy by a food sample from accelerated electrons. Therefore, in order to ensure uniform dose to a series of samples, every one of the samples must be as nearly uniform as is possible.

Nagel and Wilkins (1957) stated that sterilizing doses of ionizing radiations had no effect upon the mechanical and physical properties of mylar. Lehman and Patterson (1955) reported that extraction studies upon a condensate of dimethyl terephtalate and ethylene glycol (the type of resin of which mylar is made) under various conditions yielded essentially no extract, thus making the resin acceptable for food packaging films.

F. Bacteriological considerations

Gunderson et al. (1954) listed among the bacterial agents capable of infecting both poultry and man, Escherichia coli and the pyogenic cocci which include Staphylococcus aureus. These investigators found total viable bacterial counts of approximately 3,800 organisms per square centimeter of eviscerated poultry as it left the eviscerating plant. Walker and Ayres (1956) found viable counts of 35,000 per square centimeter on the eviscerated poultry leaving the eviscerating lines which they investigated. Gunderson et al. (1954) found viable coliform counts of 573 per square centimeter of eviscerated poultry as they left the

eviscerating line. These same investigators performed bacteriological studies upon chicken meat which was ready for a boning operation prior to the preparation of a pre-cooked chicken product. They obtained counts slightly greater than 10^5 organisms per gram at this stage.

Breed et al. (Bergey's Manual of Determinative Bacteriology - 1957) described E. coli as an aerobic and facultative anaerobic, catalase positive organism, widely distributed in nature which displays optimum growth at 30-37°C. Staph. aureus is described as an aerobic and facultative anaerobic, catalase positive organism which grows optimally at 37°C. It requires amino acids as a source of nitrogen, and thiamine and nicotinic acid for growth. Certain strains produce a potent enterotoxin which is a significant cause of food poisoning.

The use of E. coli as an index of sanitation in food products is an extension of the methods adopted by the American Public Health Association for water analysis. This has been questioned by Hunter (1939) and Appleman (1957). Both of these investigators do not deny the need of an index, but present their doubts concerning the use of coliform organisms due to their natural occurrence in many food products as well as the possible introduction through transfer of food particles, of fermentable carbon sources into the lactose media used for identification which may result in a false positive test.

Burton (1949) compared coliforms and enterococci for their relative efficiency as an index of pollution in frozen foods. He concluded that the coliform organism was the more valid index.

Dack (1956) reviewed staphylococcal food poisoning. He stated that enterotoxin production is a function of growth. Usually, hundreds of millions of organisms were present in a product which caused food poisoning, but one case was presented in which only 16 million organisms were present in the incriminated product. There is also the possibility that only a small amount of toxin need be present in order to adversely affect those people who are very susceptible.

Feig (1944) listed the food products most often incriminated in food poisoning outbreaks on a basis of the number of outbreaks. Poultry products were second only to meat products on this list.

III. DEVELOPMENT OF METHODS

A. General

The overall thesis problem required the development and evaluation of a series of methods to determine procedures by which suitable results could be obtained.

A series of samples had to be inoculated in order to observe growth phenomena. The time required to inoculate the samples of a complete series was estimated at 20 minutes. The test microorganism should not, therefore, die off in significant numbers when placed in a non-nutritive dilution blank, at a cell concentration of approximately 2×10^6 cells per milliliter, for 20 minutes. The dilution blank had to be non-nutritive since the nutritional quality of the substrate medium was to be evaluated. In view of reports in the literature concerning a rapid decrease in viable cells encountered in some dilution blanks, it was necessary to perform a series of experiments to determine whether the organisms proposed for study in future investigations would exhibit an undesirable loss of reproductive capacity.

Each of the inoculations in the series had to contain the same number of cells and had to be of 0.01 to 0.05 milliliters in size, so that it could be spread over a one square inch surface which also had to be standardized. The ability to recover the cells inoculated onto a solid substrate surface also had to be demonstrated.

In order to compare raw chicken meat with either radiation processed or heat processed chicken meat, as a growth

medium for microorganisms, it was first necessary to obtain the raw meat in an essentially sterile state. A requirement of less than ten organisms per gram was set as the level at which there would be no serious interference with the growth of the inoculated bacteria, since the intended inoculum was to contain 5,000 to 20,000 organisms and the size of the sample was to be less than five grams.

A method which could be used to evaluate the success of the aseptic technique for the removal of chicken muscle and to extend the range of standard plate count techniques into more concentrated slurries of chicken meat was required. After plate cultures of food products have been prepared in accordance with standard methods and incubation has been completed, the ease with which colonies can be recognized is dependent on the extent to which the sample is diluted. To the naked eye, the medium in which the bacterial colonies have grown may vary from total opacity to complete transparency. In cultures prepared from low dilutions of food products, bacterial colonies may be masked by food particles or food particles may appear to be bacterial colonies. The method, therefore, must distinguish between chicken particles and bacterial colonies.

The development of a simulated chicken medium to provide the ultimate in control over the surface area and the moisture content was desirable. A concentrated slurry of chicken meat solidified by a non-nutritive gelling agent resulting in an essentially unchanged nutritional condition of

the chicken meat was required.

Each of the methods developed required subsequent testing and evaluation to determine the validity of the results which might be obtained through their use.

B. Experimental

1. Methods

a. Development of the inoculation procedure

Initially an attempt was made to obtain an accurate measure of the inoculum through the use of a microsyringe.[1] The procedure was very time consuming and some difficulty was encountered in cleaning and sterilizing the syringe due to its fixed needle type of construction. The method was therefore abandoned.

The following method was next developed. Standard 1.1 milliliter milk pipettes were drawn out to a fine capillary tip. An opening which would deliver a drop of approximately the required size was obtained by breaking the tip at a suitable place. The pipette type inoculator was then fitted with a rubber bulb to enhance filling and subsequent drop delivery. Delivery of a drop was accomplished by applying gentle pressure to the bulb until the formed droplet reached dimensions of such size and weight that it overcame surface tension forces and fell freely into the sample which was being inoculated. It was assumed that a drop delivered in this manner, under uniform conditions, would be of uniform volume.

Experiments were run, using the pipette type inoculator, to determine the reproduceability of the size of a drop.

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1. Product of the Hamilton Company, Inc., Whittier, California.

Two major difficulties were encountered when inoculating pipettes were used. The reproduction of tips of uniform size in different pipettes was virtually impossible, thus making it difficult to run comparable experiments in which more than a single inoculator was required. The fragility of the fine pipette tips made the pipettes very difficult to wash and sterilize without occasional breakage. Such losses could not be tolerated when day to day experiments of a comparative nature were being run.

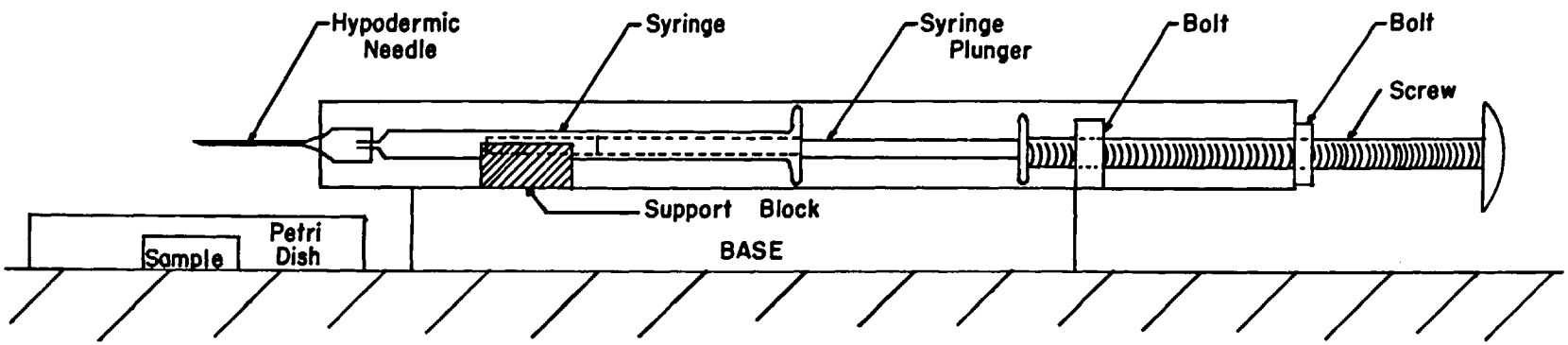
An improved technique involving the same principles as were described for the pipette inoculator was eventually developed. A sterile B-D Yale 1cc. tuberculin syringe was fitted with a sterile B-D Yale, regular point, 24 G., 3/4 in. hypodermic needle. After the syringe was filled with inoculum it was placed in a screw type device with the bevelled opening of the hypodermic needle facing downward and the head of the syringe plunger in a position from which it could be driven forward uniformly by turning the screw into its thread. A diagram of the loaded syringe is presented in figure 1. The syringe inoculator was rugged and gave comparable results when another instrument of the same type was substituted, since commercial needles and syringes of the same general size have uniform dimensions.

b. Preparation of solid substrate samples of uniform surface area

Prior to the initiation of the investigation, a method was developed which provided uniform discs of substrate. A

Figure 1

Syringe Inoculator



50 cc. syringe barrel with inside diameter of 1.096 inches, was modified by cutting off the front portion at a point as close to the zero line as was possible. The syringe barrel was then plugged at both ends with No. 6 rubber stoppers which were covered with 200 gauge saran, to prevent the rubber from fusing to the glass during sterilization. The sterile barrel was aseptically filled with sterile medium containing enough agar to provide a rigid gel upon cooling. After cooling, the solidified medium was pushed out of the syringe by use of the sterilized syringe plunger and uniform discs were cut with a sharp sterile knife in such a manner that they fell into sterile Petri dishes. The discs, so obtained, were of identical surface area, 0.94 square inches, and of reasonably uniform volume, approximately 5 cc.

After a disc was inoculated with cells, in 0.01 to 0.05 milliliters of solution, it was necessary to spread the inoculum over the entire disc surface, since growth was to be expressed on a per disc area basis. A T shaped rod was made of 1/16 inch diameter glass. The handle was approximately four inches long and the cross bar was approximately 1/4 inch long. The spreader was sterilized prior to use and was used throughout any one series of inoculations. Spreading of the inoculum was accomplished by several strokes in several directions so that the spreader rod reached all areas of the disc surface.

c. The recovery of cells from the surface of a solid substrate sample

In order to recover the cells from the inoculated disc,

complete grinding of the substrate disc to particles which would not clog bacteriological pipettes was required. To accomplish this it was necessary to modify the angle of the blades in the stainless steel blendor cups[1] which were used in the size reduction operation. The modification removed some of the curvature of the blade, thus extending its outer tips toward the walls of the blendor cup. When one disc was placed in a blendor cup with 99 milliliters of chilled water, a grinding time of four minutes was found to be fully adequate to satisfy the previously mentioned requirement.

d. Techniques used to obtain samples of sterile raw chicken muscle

Live chickens, weighing approximately five pounds each, were brought into the laboratory. The slaughtering area was pre-scrubbed with "Just"[2], a detergent soap solution having a phenol coefficient greater than 6.0. The area was allowed to dry naturally without rinsing. The chicken was slain and bled by severing the throat veins. The head and the incision were then wrapped in a sterile towel which was secured just below the incision by a rubber band. The bird was then dipped in 180°F water, which had previously been brought to a boil by direct steam injection. The bird was submerged in the water for 10 to 20 seconds after which it was placed upon the scrubbed

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1. Product of the John Oster Mfg. Co., Racine, Wisconsin

2. A product of Associated Just Distributors, Inc., Baltimore, Maryland.

bench and defeathered by hand. After the feathers were completely removed the bird was raised, the bench was again scrubbed with the soap solution, and the bird was replaced upon the bench. The bird itself was then scrubbed with the soap solution. The head wrapping was removed and replaced by a clean one. The wings of the bird were twisted behind its back so that they would not interfere in subsequent treatments. The entire bird was then wrapped in sterile towels and transported to a "low contamination" chamber. The chamber was a modified draft hood which was sealed so that the air inside of it was relatively stagnant. The surfaces of the chamber were thoroughly scrubbed with a soap solution prior to use. The air within the chamber was sterilized by ultraviolet rays provided by a pair of ultraviolet lamps[1] suspended in the chamber. The ultraviolet lamps were allowed to operate for at least 30 minutes before the chicken was placed in the chamber, and continued to operate during the entire experiment. The front of the chamber was closed by a sliding glass panel which was raised to a level just high enough to allow the performance of the operations which were required.

The bird was placed upon its back and the towels were turned back so that they rested on the surface of the chamber. The exposed portion of the bird was rinsed with sterile water and then scrubbed with Phisohex[2], a synthetic detergent contain-

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1. 15 watt germicidal lamps manufactured by the General Electric Co., Schenectady, N.Y.

2. Manufactured by Winthrop Laboratories, New York 18, N.Y.

ing hexachlorophene. The bird was then re-covered with sterile towels and the Phisohex was allowed to remain in contact with the skin for five minutes.

The bird was then uncovered, rinsed with sterile water, and treated with Zephiran[1], a quaternary ammonium compound. In this operation a 1:1000 aqueous solution of Zephiran was poured over the exposed portions of the bird, after which it was again covered. The Zephiran was allowed to remain in contact with the skin for ten minutes. The bird was now ready for the aseptic removal of the muscle.

The muscle desired was the pectoralis major, which is the large muscle present in the breast portion of the chicken.

All of the instruments used were previously sterilized by heat. There was frequent instrument change during the excision of each muscle to prevent any cross contamination which might occur from an instrument which had become contaminated.

The bird was uncovered and the skin, at the breast bone, was raised with a pair of forceps. The skin was then cut along the bone line and was pulled back with the forceps. The skin was removed from the breast and also from the inner portion of the leg. The pectoralis major muscle was then removed from each side of the breast. The excised muscles were placed in large sterile Petri dishes[2] to await subsequent exper-

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1. Manufactured by Wintrop Laboratories, New York 18, N.Y.

2. 150 mm. diameter x 20 mm. height.

imental procedures.

e. A method to facilitate the recognition of bacterial colonies in the presence of large numbers of food particles

Each culture of plated food material, after proper incubation, was removed from the incubator and was flooded with two milliliters of a 0.1% aqueous solution of 2,3,5 triphenyltetrazolium chloride (TTC). The flooded plate was gently rocked back and forth and from side to side several times so that the entire surface was well covered by the solution. The excess solution was poured off, the Petri dish cover replaced, the plate inverted, and allowed to remain at room temperature for 3 to 5 hours. The TTC solution diffused through the agar in the Petri dish during the first hour. The metabolizing bacterial colonies normally reduced the indicator solution within the first hour. The additional time used, allowed the more slowly metabolizing colonies to reduce the TTC solution and also resulted in color intensification within all of the colonies. All of the bacterial colonies were now colored red while particles of chicken in the medium retained their natural color.

A Quebec colony counter was found to be satisfactory for counting the colonies except in the case of extremely opaque media. When the latter situation existed a more powerful light source was required to penetrate the growth medium. Exposure of the Petri dish culture to an uncovered 100 watt lamp proved to be satisfactory for this purpose.

The method described above has been entitled, "The

Tetrazolium Flooding Technique*, and is referred to in this manner throughout the remainder of this thesis.

f. The development of a simulated chicken medium and a technique for the preparation of uniform discs of whole chicken meat

A simulated raw chicken medium was prepared by suspending a large quantity of chicken meat in a medium which would form a gel within a short time, thus preventing the settling out of the chicken solids. Approximately 30% chicken meat was considered to be the maximum concentration of solids which could be suspended since it required approximately five minutes of grinding to obtain a uniform suspension when this amount of chicken was present and a grinding period of more than five minutes would produce a temperature rise which would yield a product more cooked than raw. The preparation of this medium would yield a system in which the water activity and the surface area would be relatively constant. Therefore, any bacterial growth differences noted could be attributed to the nutritional differences of the medium being tested.

A slurry consisting of approximately 1/3 raw sterile chicken meat, 1/3 distilled water and one-third 3% solution of Purified agar was prepared. This was poured into the previously described (Page 31) open end 50 ml. syringes and then placed in the refrigerator to hasten solidification. Discs were then cut with a sterile knife, after forcing five cc. portions from the syringewith the syringe plunger.

In order to prepare discs of whole chicken meat which would be of reasonably uniform surface area, a cutting tool was

prepared. A piece of round brass stock was bored out until the diameter of the opening was exactly the same as that of the 50 ml. syringe (1.09 inches). The outside of the brass die was then bevelled to form a sharp edge. This die was always sterilized by dry heat. By placing the sterile die upon a piece of sterile chicken muscle and pressing down gently the outline of the disc was formed in the chicken. With the die still held in place, a sterile scalpel was used to cut around the die. When the die was then lifted off of the cutting surface the disc of chicken meat was usually raised with it. Sterile forceps were used to push the disc out of the die into a waiting sterile Petri dish. Each disc was placed in a different Petri dish.

For the performance of radiation studies with an electron accelerator as the radiation source, a disc of chicken meat of uniform thickness was required to ensure uniform energy absorption during the radiation exposure. This was accomplished in the following manner. Plastic Petri dishes were packaged in mylar and sterilized by treatment with 5×10^6 rad of ionizing radiations. A piece of chicken muscle was placed in an inverted sterile plastic Petri dish cover. The outside surface of the Petri dish bottom was then placed on top of the piece of chicken in the Petri dish cover. A gentle downward pressure was then placed upon the Petri dish bottom while a very sharp sterile knife was passed between the bottom and the lip of the cover with the flat of the knife blade supported by the lip of the cover which served as a guide. The piece of

trimmed-off meat was then removed with forceps leaving a piece of sterile meat which was approximately 1/4 inch thick and fairly uniform throughout.

C. Evaluation of Methods

1. Procedures

a. Survival of microorganisms in dilution blanks

Two organisms of public health significance, namely: Escherichia coli ATCC 9637 and Staphylococcus aureus ATCC 9664, were chosen as test organisms.

The age of the culture, nature of the dilution blank, temperature of the dilution blank, and effect of washing the cells were the factors considered in the studies with E. coli. The only variable considered in the studies involving S. aureus was the age of the culture since survival under the conditions investigated was satisfactory.

The bacteria were maintained on Stock Culture agar (Difco) slants at refrigerator temperature. Prior to the start of any experiment a flask containing 100 ml. of broth, pretempered to 37°C, was inoculated from the stock slant and allowed to incubate at 37°C for 24 hours. E. coli was grown in Tryptone Glucose Extract broth. S. aureus was propagated in Brain Heart Infusion (Difco).

The test cultures were prepared by transfer of three loopfuls from the 24 hour culture into 100 ml. of sterile broth, pretempered to 37°C, followed by incubation at 37°C. for the desired period of time. Experimental results were obtained through the use of standard plate count techniques. The plating medium was Tryptone Glucose Extract agar (Difco) for E. coli and Brain Heart Infusion agar (Difco) for Staph. aureus. Petri dishes were incubated at 37°C for 24-36

hours after which colonies were counted with the aid of a Quebec Colony Counter.

Washed cells were prepared by separating them from the growth medium in a high speed centrifuge[1], pouring off the liquid, resuspending the cells in physiological saline, and subsequent repetition of the procedure until the cells were washed twice with saline. The cells were then resuspended in a volume of saline equivalent to that volume of broth from which they were originally removed. Unwashed cells were removed directly from the incubated broth suspension.

All of the water used was distilled water. Saline dilution blanks were were prepared in accordance with the Recommended Methods for the Microbiological Examination of Foods (1958). All dilution blanks were pretempered to the temperature of the experiment in which they were to be used.

A temperature of 10°C was obtained by use of an electric refrigerator. Room temperature was considered to be 30°C. A constant temperature incubator was used for the experiments which were carried out at 37°C.

The cell suspensions which were sampled for survival, when prepared, contained approximately two million organisms per milliliter. This was the actual suspension density which was required in the growth studies which were to take place subsequent to the survival studies.

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1. Servall superspeed angle centrifuge Model SS-1 operated at 11,950 rpm. Manufactured by Ivan Sorvall, Inc., Norwalk, Conn.

The experiments were designed as screening experiments and all results obtained initially were the results obtained with single samples plated in duplicate. In those tests in which the results appeared to satisfy the requirements of the experiments, further tests were made to determine whether the data could be duplicated.

Survival studies of unwashed cells from both an 18 hour culture of E. coli and a 24 hour culture of S. aureus at 10°C in distilled water were carried out on four different days. Samplings were made at 0 time and after 60 minutes in the experiments with E. coli, and at 0 time and after 40 minutes in the experiments involving S. aureus. The dilution conditions chosen for ultimate use were sampled at ten minute intervals for the time periods mentioned above, to ensure that the end result was representative of the entire survival picture.

The results of this series of experiments are presented in Tables 1 through 3.

b. Inoculation procedure and recovery of microorganisms from the surface of solid media

The test organism selected for these studies was Escherichia coli ATCC 9637. Stock cultures of the organism were maintained on Stock Culture agar (Difco) at refrigerator temperature. Prior to the start of any experiment the organism was transferred by use of an inoculating needle, from the stock slant to a flask containing 100 milliliters of sterile, tempered (37°C) Tryptone Glucose Extract (TGE) broth which was then incubated at 37°C for at least 24 hours, but not longer than

30 hours. Eighteen hours before an experiment was to begin three loopfuls of the 24-30 hour culture were transferred to a flask of sterile, tempered TGE broth and incubated at 37°C.

All experimental measurements were made by use of standard plate count techniques. The plate culture medium used was TGE agar (Difco). Incubation was carried out at 37°C for 18-24 hours.

The dilution blanks used throughout the experiments were sterile chilled distilled water. Chilled distilled water containing a suspension of unwashed cells was used as inoculum. The inoculum was adjusted to contain 3,000 to 40,000 cells in a volume of 0.1 to 0.5 milliliters.

The previously described inoculator pipette (Page 31) was filled with a cell suspension from a 1/100 dilution of an 18 hour culture of E. coli in TGE broth at 37°C. A series of dilution blanks, each containing 99 milliliters of chilled distilled water were inoculated, each with one drop of cell suspension. Plate cultures were then prepared from proper decimal dilutions.

Using the already filled inoculator pipette, a series of TGE agar discs prepared in the manner described earlier in the methods section (Page 31), were inoculated, each with one drop of cell suspension. The drops were then spread upon the surface of the discs in accordance with the method also previously presented (Page 33).

The discs were then transferred to sterile stainless steel Oster blendor cups with modified blades (as described

elsewhere). Ninety-nine milliliters of chilled water was added to the blendor cup and the cover was set in place. The cup and contents were then placed on a Waring blendor base[1], which was modified to hold the Oster cup, and ground for four minutes. Plate cultures were then prepared from the proper decimal dilutions.

Runs 1, 2, and 3 were performed simultaneously with all of the inoculum drops coming from the same subculture. Plate cultures were made in triplicate. Runs 4 and 5 were performed simultaneously with both of the inoculum drops being derived from a subculture different from the one used in Runs 1, 2, and 3. In these last two runs, seven plate cultures were prepared from each sample. After incubation of the plate cultures for 18-24 hours at 37°C, the colonies were counted with the aid of a Quebec Colony Counter.

The results which are presented in Table 4 were analyzed statistically for homogeneity of variance and equality of means. The plate count data for drops inoculated directly into dilution blanks and drops inoculated first onto the surface of discs and then transferred into dilution water through a grinding operation, were compared for homogeneity of variance and equality of means. The means were tested by an analysis of variance test for two variables of classification with repeated measurements.

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1. Manufactured by Waring Products Corporation, New York 36, N.Y.

c. The recognition of bacterial colonies in the presence of large numbers of food particles

The first seven samples were excised from the same bird. The pieces of chicken meat, from the pectoralis major muscle, were obtained in the manner described previously (Page 34). The pieces weighed 4.83, 11.50, 9.05, 5.50, 7.81, and 12.20 grams, respectively.

The following tests were made in determining the level of contamination of the "sterile" chicken muscle:

Samples 1, 2, and 3; Immediately after excision pieces of chicken were placed in sterile blendor cups with 99 milliliters of chilled sterile distilled water and were blended for four minutes. One milliliter portions were then plated in triplicate.

Samples 4 and 5; Pieces of chicken were placed in a 37°C incubator for six hours prior to being blended in 99 milliliters of chilled sterile distilled water for four minutes. One milliliter portions were then plated in duplicate.

Samples 6 and 7; Pieces of chicken were incubated at 37°C for 30 hours prior to being placed in blendor cups with 99 milliliters of water and blended for four minutes. One milliliter portions were plated in triplicate.

Sample 8; 124.9 grams of chicken meat was blended with 198 milliliters of chilled sterile distilled water for four minutes. One milliliter portions were plated in quadruplicate.

Sample 9; 116.4 grams of chicken meat were blended in 99 milliliters of water for four minutes. Six one milliliter

portions were plated.

Sample 10; 91.9 grams of chicken meat were blended with 99 milliliters of chilled sterile distilled water for four minutes. Three grams of the resulting slurry were weighed into each of eight replicate plates.

Sample 11; 66.4 grams of chicken meat, 93.0 grams of molten 3% Purified agar (Difco), and 57.1 grams of sterile distilled water were placed in a blendor cup and ground for five minutes. A total of 110 grams of the resulting slurry were poured into five Petri dishes.

Sample 12; 101.0 grams of chicken meat and 102.0 grams of chilled sterile distilled water were placed in a blendor cup and ground for five minutes, after which 92.3 grams of 3% molten Purified agar were added. The mixture was then blended for one minute. One hundred twenty-eight grams of the resulting slurry were distributed among six Petri dishes.

Sample 13; 37.3 grams of chicken were ground for six minutes in 42.5 grams of chilled sterile distilled water. Ninety-four and five-tenths grams of sterile 3% Purified agar solution were then added and the three components were blended for one minute. One hundred and fifty grams of the slurry were then apportioned into six Petri dishes.

The nutrient medium added to all of the Petri dishes was Tryptone Glucose Extract agar (Difco). Samples 1 through 10 were poured in the conventional pour plate method. The plates from samples 11 through 13 were overlaid with a layer of

the TGE agar.

All of the Petri dishes were incubated at 37°C for 24 hours. The plates were then counted with the aid of the Tetrazolium Flooding Technique. The results are presented in Table 10.

The following tests were made in order to determine the improvement in accuracy which resulted from the use of the Tetrazolium Flooding Technique.

Discs of sterile meat of approximately one square inch surface area and 1/4 inch thickness were prepared and inoculated with approximately 10,000 cells of an 18 hour culture of E. coli. The discs were incubated at 37°C. After various periods of incubation, discs were selected, removed from the incubator, and placed in a sterile blendor cup together with 99 milliliters of sterile chilled distilled water. After grinding for four minutes a series of dilutions were plated using TGE agar as the nutrient medium. The dishes were incubated at 37°C.

After 24-36 hours the plates were removed from the incubator, the discernible colonies were counted using a Quebec Colony Counter, and the results were recorded. The plates were then subjected to the Tetrazolium Flooding Technique, after which they were re-counted and the new results were recorded. Comparison of the two sets of data are presented in Table 9.

d. The simulated chicken model system

Solidifying agents were investigated in order to determine their contribution of nutrients to the model system. The following gelling agents were considered:

1. Bacto agar - A purified agar from which the extraneous matter, pigmented portions and salts are reduced to a minimum. (Difco Manual 1953).

2. Seakem type 204 - A mixture of Irish Moss Extractive, locust bean gum, and edible potassium salts. (Product of Seaplant Chemical Corporation, New Bedford, Massachusetts.)

3. Purified agar - Bacto agar repeatedly washed with tap water, distilled water, pyridine, and alcohol to remove soluble salts, nitrogenous compounds, and vitamins. (Difco letter 1957.)

All of the gels were prepared at 3% solutions in distilled water. After sterilization, discs were prepared in the manner previously described (Page 31). The discs were inoculated with one drop of a 1/100 dilution of an 18 hour culture of E. coli grown in TGE broth using the inoculating syringe. The inoculated discs were placed in a 37°C incubator within a chamber in which there was a layer of water below the plates, in order to maintain high humidity conditions during the incubation period.

At selected sampling times, duplicate discs were removed from the incubator, placed in sterile blendor cups with 99 milliliters of chilled sterile distilled water and blended for four minutes. Proper decimal dilutions were plated in

triplicate using TGE agar as the plating medium. The plates were incubated at 37°C for 24 hours prior to counting of the formed colonies. The results of the studies are presented in Figure 2.

The Purified agar was subjected to further testing for the presence of toxic substances. Discs of 3% Purified agar were placed in Petri dishes. They were then covered with TGE agar which was previously inoculated with 10^5 cells per milliliter of E. coli. The plates were then incubated at 37°C for 24 hours, after which they were observed for areas of inhibition.

The simulated raw chicken was compared with whole raw chicken by subjecting both to a growth study utilizing E. coli as the test organism. Since there was a short 45°C heating period involved in the preparation of the simulated raw chicken, whole raw chicken was subjected to a short 45°C heating period, after which the growth of E. coli was studied upon this medium.

Simulated chicken was prepared by placing 66.4 grams of raw sterile chicken meat in a blendor cup, adding 57.1 grams of chilled sterile distilled water, and 93.0 grams of sterile 3% Purified agar solution at 45°C. The three components were blended for five minutes. The open end syringes were then filled, placed in the refrigerator to solidify, and the discs were prepared as previously described (Page 33).

Whole chicken discs were prepared by means of the sterile die method which was described previously (Page 39). Subjection of whole chicken discs to 45°C was accomplished by

placing the discs in prewarmed Petri dishes and then placing the dishes in a 45°C air oven for 20 minutes, after which the dishes and discs were transferred to a refrigerator for 20 minutes prior to inoculation.

All discs were inoculated with one drop from a 1/100 dilution of an 18 hour culture of E. coli grown in TGE broth at 37°C. The inoculated discs were incubated at 37°C in the humidity chamber (See Page 49). At selected times, duplicate or triplicate samples were removed, placed in a blendor cup together with 99 milliliters of chilled sterile distilled water and ground for four minutes. Decimal dilutions were then plated in triplicate with TGE agar as the nutrient medium. After 24 hours incubation at 37°C, the plates were removed from the incubator, subjected to the Tetrazolium Flooding Technique, and counted. The results are presented graphically in figure 3.

C. Evaluation of Methods

2. Results

a. Survival of microorganisms in dilution blanks

Examination of Table 1 shows that washed cells of E. coli were unable to survive a 60 minute standing period in distilled water at 37°C, 30°C, or 10°C. Survival at 10°C appeared to be somewhat greater than that at 30°C and greater survival was indicated at 30°C than at 37°C. At both 30°C and 37°C there was a trend toward increased survival with increased age of the culture. At 10°C no such trend was evident. Complete survival of washed cells of E. coli was observed in physiological saline at 30°C independent of the age of the culture when cultures between 11 and 25 hours old were examined. Unwashed cells of E. coli exhibited complete survival in distilled water at either 10°C or 30°C and at all culture ages between 14 and 24 hours.

The data in Table 1 also demonstrates that unwashed cells of S. aureus suspended in chilled distilled water were more susceptible when the culture was 14 hours old than they were when the cells were either 18 or 24 hours old. There seemed to be a small amount of reproduction in the case of the 18 hour cultures. Tables 2 and 3 contain the results of repeated experiments evaluating the survival characteristics of E. coli and S. aureus at short intervals during the holding time in dilution blanks. The average results for unwashed cells of an 18 hour culture of E. coli suspended in chilled distilled water showed a high degree of stability during the entire test period while those for a 24 hour culture of S. aureus

suspended in chilled distilled water exhibited an oscillating effect which was not considered serious enough to interfere with future experiments.

b. Inoculation procedure and recovery of microorganisms from solid surfaces

It is evident from the data presented in Table 4 that there was some variation, both between plate counts from a single drop and between plate counts from successive drops, whether they were derived from a single subculture or from more than one subculture. The variations appear to be in the same order of magnitude for both the directly inoculated drops and those which were recovered from the surface of agar discs.

In order to substantiate the hypothesis that the errors within a single sample, which were attributable to experimental error, were as great as or greater than the errors between samples, which were attributable to drop size variation, the data from each of the two major categories were subjected to an analysis of variance test. The hypothesis tested was: The five groups of observations were all drawn from populations with the same mean or there was no difference between means as a result of a difference in treatment.

A test which was developed by Box (1949) was used to evaluate the hypothesis of equal variance within samples. The results of this analysis are presented in Table 5.

The critical value of F at the 1% significance level with 4 and 280 degrees of freedom is a value greater than 3.32 and the critical value with 4 and 22 degrees of freedom is 4.31. The experimental value obtained were 0.486 and 0.492,

Table 1

Effect of age of the culture and environmental conditions upon the survival of E. coli and Staph. aureus during a post dilution standing period

Age of the culture (hours)	E. COLI Per cent surviving						S. AUREUS
		Washed			Unwashed		Unwashed
	Saline	Water			Water		Water
	30°C	10°C	30°C	37°C	30°C	10°C	10°C
11	96						
14	98					102**	83**
16	106	64	36	29	91		
17	103	52			117		
18	92	79	32		100*	102***	146**
19	90						
20	89					114	
21						105	
22	98	58	68	47			
24		74	60			96**	101**
25	101						

*Standing period of 30 minutes
**Standing period of 40 minutes
***One sample of the four averaged for this value had a standing period of 40 minutes

Table 2

Survival of unwashed cells from an 18 hour culture of E. coli* in distilled water at 10°C

Time after dilution (minutes)	Per cent survival				
	Run 1	Run 2	Run 3	Run 4	Ave.
0	100	100	100	100	100
10	99	102	103	98	100
20	100	100	105	98	100
30	96	101	116	108	105
40	101	89	111	95	99
50	108	103	104	-	105
60	105	96	112	-	105

*Cells propagated in Tryptone Glucose Extract broth (Difco) at 37°C.

Table 3

Survival of unwashed cells from a 24 hour culture of Staph. aureus* in distilled water at 10°C

Time after dilution (minutes)	Per cent survival				
	Run 1	Run 2	Run 3	Run 4	Ave.
0	100	100	100	100	100
10	89	93	70	93	86
20	87	107	94	121	102
30	83	83	92	96	88
40	93	100	-	118	104

*Cells propagated in Brain Heart Infusion (Difco) at 37°C.

respectively; therefore, the indication was that equal variance existed within samples.

The results of the individual analysis of variance for the two sets of data are presented in Table 6. The critical F value at the 5% significance level with 4 and 18 degrees of freedom is 2.93, while with 4 and 16 degrees of freedom it is 3.01. The experimental F values were 1.667 and 1.008, neither of which were in the critical regions. The reproduceability of a drop formed and released by comparable implements was therefore demonstrated.

Tables 7 and 8 present the results of the statistical comparisons between drops inoculated directly into dilution blanks and drops inoculated onto the surface of agar discs, with subsequent suspension in dilution water by comminution. The hypothesis of equal variance was tested by means of a two tailed test for the comparison of two samples. The critical region at the 1% significance level was values of F greater than 3.22 and less than 0.314. The experimental F value of 0.6909 was not in the critical region; therefore the hypothesis of equal variance of both samples was accepted. The statistical results are presented in Table 7. Table 8 is the analysis of variance table which resulted from the test for equality of means between the directly diluted drops and the drops which were placed upon the surface of agar discs prior to their dilution. The test for significant interaction resulted in an F value of 1.35, which when compared to the critical value of 2.61 at the 5% level of significance with 4 and 34 degrees of freedom

led to the conclusion that there was no significant interaction. Row effects and column effects also proved insignificant with F values of 0.074 and 1.280 versus critical values of 4.08 and 2.61, respectively at the 5% significance level. The ability to recover 100% of the cells from a substrate disc was therefore demonstrated.

c. The recognition of bacterial colonies in the presence of large numbers of food particles and the level of contamination of the "sterile" chicken muscle

The results presented in Table 9 show that in every case the number of colonies observed after the application of the TTC was greater than that observed prior to the treatment. The variations were from 4.2% to 36.8% with the greater variations generally occurring in the lower dilutions but not in every case.

The plates prepared in the course of testing chicken muscle for sterility were, in most cases, uncountable prior to the application of the Tetrazolium Flooding Technique due to the high concentrations of chicken meat present in them. The plates of samples 11, 12, and 13 were totally opaque with no visible colonies until after the TTC treatment and subjection of the plates to the uncovered lamp counting method. The results obtained through the use of the Tetrazolium Flooding Technique are presented in Table 10.

The results in Table 10 indicate that only three samples of the first seven failed to meet the required limit of ten organisms per gram. Only one of these, however, was serious

Table 4

Plate counts of one drop of inoculum of E. coli: a) placed directly into a 99 milliliter dilution blank and b) placed first upon an agar disc followed by suspension in a 99 milliliter dilution blank

Experiment number	Dilution blanks Plate counts								Agar discs Plate counts						
	1	2	3	4	5	6	7	Ave.	1	2	3	4	5	6	Ave.
1	68	67	58					64.33	54	66	72				64
2	67	77	76					73.33	63	51	73				62.3
3	73	68	63					68.00	59	71	60				63.3
4	83	75	80	58	68	79	60	71.86	62	81	72	76	93	60	74
5	79	70	70	62	56	59	64	65.71	79	71	83	71	76	65.	74.2

Table 5

Test for homogeneity of variance between groups of observations in Table 4

Hypothesis	Variances of 5 normally distributed samples are equal	Direct inoculation	Disc Inoculation
Significance level	.01		
Statistic	$F = \frac{f_2 M}{f_1(\bar{u}-M)} = \frac{614.508}{1263.607}$	0.486	0.492
Sampling distribution	$F(f_1, f_2)$	F(4, 280)	F(4, 22)
Critical area	reject if $F > F_{.99}(f_1, f_2)$	>3.32	4.31

Table 6

Analysis of variance to test for equality of means of groups of observations in Table 4

	Sum of squares	Degrees of freedom	Mean square	F ratio
		Direct Inoculation		
Means	255.24	4	63.81	$F = \frac{63.81}{63.31} = 1.008$
Within	1139.63	18	63.31	$F_{.95}(4,18)=2.93$
Total	1394.87	22		
		Disc Inoculation		
Means	610.973	4	152.7433	F=1.667
Within	1446.17	16	91.6356	$F_{.95}(4,16)=3.01$
Total	2077.143	20		

Table 7

Test for homogeneity of variance between drops of inoculum placed directly into dilution water and drops of inoculum placed upon substrate discs and then into dilution water*

Hypothesis:	Variances of two normally distributed grouped samples are equal	
Significance level	.01	
Statistic	$F = S_1^2/S^2_2$	0.6909
Critical region	$F_{99}(22,20)$	>3.22
	$1/F_{99}(20,22)$	<0.314

*Data in Table 4.

TABLE 8

Analysis of variance to test for equality of means between directly diluted drops of inoculum and those first placed upon a substrate surface*

	Sum of squares	Degrees of freedom	Mean square	F ratio
Row means	5.987	1	5.987	
Column means	412.232	4	103.058	$F=\frac{103.495}{76.6412}=1.3504$
Interaction	453.981	4	103.495	$F_{95}(4,34)=2.61-2.69$
Subtotal	872.2	9	96.9	
Within groups	2605.8	34	76.6412	
Total	3478.0	43		
Rows	5.987	1	5.987	$F=\frac{5.987}{80.5206}=0.0744$
				$F_{95}(1,38)=4.08-4.17$
Columns	412.232	4	103.058	$F=\frac{103.058}{80.5206}=1.2799$
Residual	3059.781	38	80.5206	$F_{95}(4,38)=2.61-2.69$
Total	3478.000	43		

*Data in Table 4

Table 9

Plates of chicken samples, inoculated with E. coli, before and after treatment with 2, 3, 5 triphenyltetrazolium chloride (TTC)

Dilution	Number of Petri dishes	No. of colonies observed prior to flooding with TTC	No. of colonies observed 3-5 hrs. after flooding	Per cent difference[1]
10^{-1}	2	413	458	9.8
10^{-2}	1	48	76	36.8
10^{-2}	2	414	473	12.5
10^{-2}	2	619	646	4.2
10^{-3}	2	132	189	30.2
10^{-3}	4	869	934	7.0

[1] $\frac{\text{Number after treatment - number before treatment}}{\text{number after treatment}} \times 100$

Table 10

Contamination of aseptically excised chicken muscle

Experiment number	Quantity of chicken plated (grams)	Plate counts	Organisms per gram
1	0.51	60, 72, 72	409.8
2	0.46	0, 1, 1	4.3
3	0.23	0, 0, 0	0
4	0.16	2, 0	12.5
5	0.23	0, 0	0
6	0.25	0, 2, 1	12.0
7	0.52	0, 1, 1	3.8
8	2.23	6, 5, 3, 1	6.7
9	3.24	2, 3, 1, 1, 3, 2	3.7
10	11.50	3, 2, 6, 2, 2, 3, 5, 5	2.4
11	30.3	12, 5, 5, 9, 11	1.4
12	43.8	0, 3, 4, 4, 2, 9	0.5
13	32.1	2, 2, 1, 0, 3, 3	0.3

enough to cause concern. The incubated samples did not contain any more bacteria than were present upon those samples which were plated immediately after excision.

The later experiments represent a trend toward lower counts as the methods became more routine.

d. The growth of Escherichia coli on various gelling agents

Each point on the curves presented in figure 2 is representative of the logarithm (base 10) of the mean number of viable organisms recovered from the surface of two agar discs, from which triplicate plate cultures were originally prepared and mean counts obtained.

There was growth of E. coli through almost five log cycles upon the 3% Seakem type 204 gel and growth through three log cycles on 3% Bacto agar gel. There was no growth on 3% Purified agar gel. In fact, there was a steady decline of viable cells throughout the experiment.

The seeded agar toxicity test which was performed upon the Purified agar yielded negative results with no indication of either inhibition or stimulation of the organisms in direct contact or in the vicinity of the Purified agar discs.

The early portions of the Bacto agar and the Seakem type 204 growth curves are presented as dotted lines (figure 2) since no points within this region were obtained. The initial point for all media was taken as that determined for the Purified agar since all were inoculated at the same time.

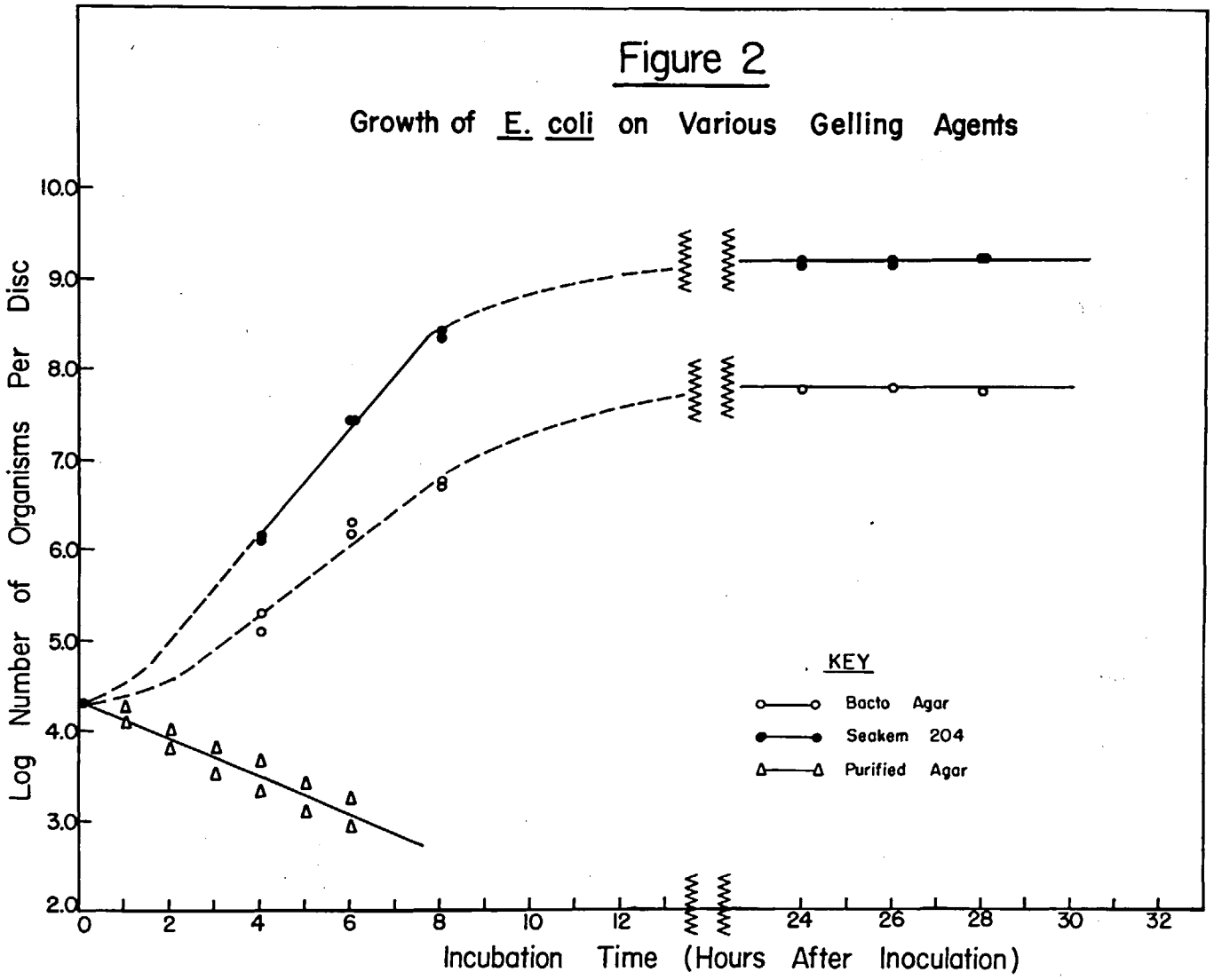


Figure 2
Growth of E. coli on Various Gelling Agents
Log Number of Organisms Per Disc
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
0
2
4
6
8
10
12
24
26
28
30
32
Incubation Time (Hours After Inoculation)
KEY
Bacto Agar
Seakem 204
Purified Agar

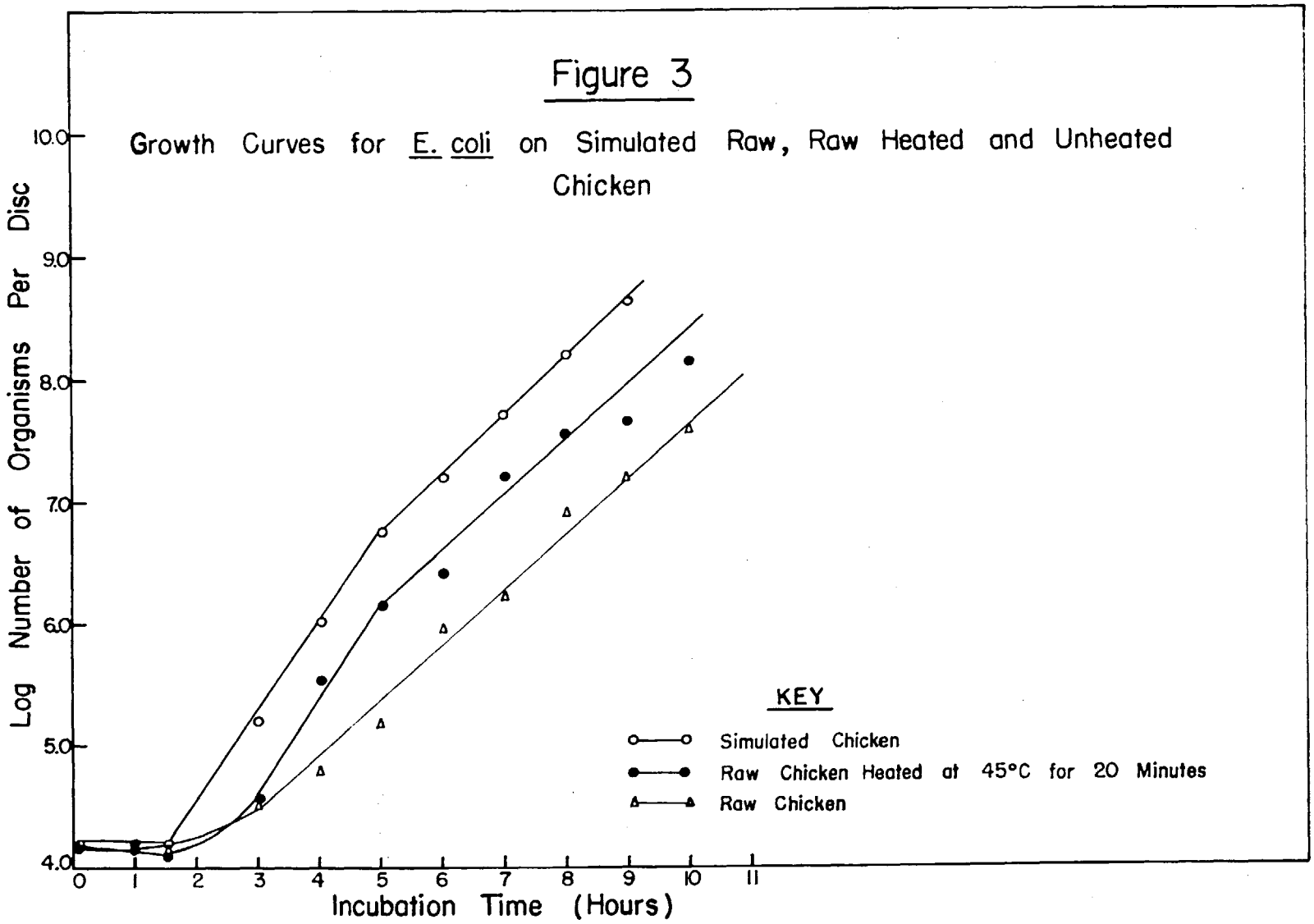
e. The comparison of growth phenomena of E. coli upon raw chicken, simulated raw chicken, and raw chicken treated with mild heat

Each point on the growth curve for E. coli on raw whole chicken, which is presented in figure 3, represents the log of the mean number of viable organisms recovered from the surface of three discs, from which triplicate plate cultures were originally prepared and mean counts obtained.

Each point of the two remaining curves of figure 3 are representative of the log mean of two discs, the means of which were determined from three replicate plate counts.

The variations of adjustment period growth between the simulated chicken and the two whole chicken samples was considerable. The simulated chicken medium allowed the organisms to enter the logarithmic phase of growth within the first 90 minutes, whereas in the case of the whole chicken media the logarithmic growth phase was not initiated until approximately 180 minutes had elapsed. Another difference observed was the rapid multiplication rate at the onset of the log phase in both the simulated chicken medium and the whole chicken which had been heated, followed by a period of growth at a rate approximately equal to that which was observed from the beginning of the log phase when growth was carried out on whole chicken medium.

Figure 3


Growth Curves for E. coli on Simulated Raw, Raw Heated and Unheated Chicken
Log Number of Organisms Per Disc
10.0
9.0
8.0
7.0
6.0
5.0
4.0
0
1
2
3
4
5
6
7
8
9
10
11
Incubation Time (Hours)
KEY
Simulated Chicken
Raw Chicken Heated at 45°C for 20 Minutes
Raw Chicken

C. Evaluation of Methods

3. Discussion

a. Survival of microorganisms in dilution blanks

The degree of survival requisite to the successful conduction of the experiments which were to follow was in the range of 90 to 100%; therefore, anything less than this degree of survival was considered to be unsatisfactory. The time period during which this degree of survival was required was 20 minutes. Therefore, the results of tests which showed adequate survival after 40 and 60 minutes in dilution blanks included a safety factor of some proportions.

The poor survival characteristics of the washed cells of E. coli supported the reports of Lamanna and Mallette (1953) and Winslow and Falk (1923) since the pH of the sterilized distilled water used was somewhat below that which was found to be optimum. These results did not agree with those of Wilson and Brooke (1927) who reported complete survival of washed cells of E. coli in distilled water. The apparently conflicting data may be the result of minor variations in the distilled water from laboratory to laboratory, as it appears that small amounts of impurities exert a vast influence upon the ability of an organism to survive.

The transfer of one milliliter of cell suspension in broth to 99 milliliters of distilled water provided complete protection for E. coli and S. aureus, as might be predicted from the data of Winslow and Brooke (1927) who reported protection of several organisms in distilled water when one part in 100 of broth was added.

b. Inoculation procedure and recovery of microorganisms from the surface of solid media

The application of statistics to microbiological problems is based upon the major assumption that within a population the organisms are normally distributed. This assumption must be accepted, a priori, since there are very few statistical methods available for the analysis of data which are derived from populations which are not normally distributed.

The test of significance in the analysis of variance is only valid if the observations are from normally distributed populations and if the variance of each group of observations is the same. Dixon and Massey (1951) state that investigation has shown that the results of the analysis of variance are changed very little by moderate violations of the assumptions of normal population and equal variance. In view of this statement it would seem that a test for the homogeneity of variances may be required but that it would be necessary to use a significance level which ensures that rejection of a true hypothesis is rare. If the hypothesis of equal variance were rejected, validity of the analysis of variance test would be doubtful. The consequence of rejecting a true hypothesis in this case is very serious since it invalidates the analysis of variance test; while the acceptance of a false hypothesis occasionally would not disturb the validity of the results significantly since it was pointed out above that moderate violations of equal variance are essentially inconsequential.

Bartlett (1937) developed a test whereby the hypothesis of equal variance could be tested for a group of samples provided

the number of observations within a group was four or more. The method was modified by Box (1949) so that it could be used when there were less than four observations within a group. Box's method was recommended for use by Dixon and Massey (1951).

The analysis of variance was performed in accordance with the procedure outlined by Dixon and Massey (1951). The requirement of equal means for comparison of day to day data make the acceptance of a false hypothesis very undesirable since the initial point, which is being tested here, exerts a profound influence on all of the points which will be obtained as a growth experiment progresses. It appeared as though it would be of less consequence to occasionally reject a true hypothesis than it would be to occasionally accept a false one. Therefore, a significance level of 5% was chosen.

The tests for homogeneity of variance between two samples and equality of means with two variables of classification and repeated measurements were performed in accordance with methods outlined by Dixon and Massey (1951). The significance levels selected were the same as those used in the tests previously discussed.

The insignificant interaction term of the two variable variance analyses showed that the means were no more disperse than might be expected from a series of samples from the same population.

When both the row and column effects proved insignificant there was good evidence that the variations caused by the population variation and experimental errors were greater than those

caused by the two variables which were under consideration. The insignificance of the interaction term showed that there were no uncontrolled experimental factors of great enough magnitude to influence the experimental results significantly.

The symbols employed in the statistical analysis are those of Dixon and Massey (1951) and Hald (1952).

A detailed description of the statistical methods used together with a set of sample calculations may be found in Appendix II.

c. The sterility of raw chicken muscle

If the data of Walker and Ayres (1956) is applied to the time between poultry leaving the eviscerating plant and its appearance in the retail market, the poultry would certainly contain an amount of contamination which would render it useless for the purposes of these studies. If sterilization studies alone were contemplated this contamination would not be of great concern, but the experimental design called for a comparison of raw chicken meat with heat processed and radiation processed meat. The radiation processing was to take place at both sterilizing and pasteurizing levels.

Absolute sterility was not established as a requirement since it was believed that less than fifty contaminating organisms in the presence of 5,000 inoculated bacteria would not add appreciably to the error of the experiments. The added precautions which would be required and the added efforts which would have to be expended in order to achieve absolute sterility were not commensurate with the benefits which would be gained therefrom.

The trend toward lower counts in later experiments was indicative of the value gained from experience in an operation which is so much an art as was this one.

Several of the experiments planned for the future required an incubation period prior to inoculation with the test microorganisms. In order to perform these experiments it was anticipated that extreme pains would have to be taken to obtain absolutely sterile chicken meat. The results obtained in this series of experiments tended to show that even though there were a few organisms present during the incubation period, there was no evidence of multiplication during that time. This was not the result of the incubation temperature since these same organisms formed colonies at the very same temperature when they were subcultured in TGE agar. The failure to multiply may have been the result of a lack of some essential nutrient which was not available on the raw chicken or it could also have been the presence of a natural inhibitor in the raw chicken which was subsequently diluted out during the plating procedure. In any case, the knowledge that the presence of a few organisms would not be likely to cause a serious contamination problem in experiments requiring incubation periods prior to inoculation, was reassuring.

These results are in good agreement with data presented by Tanner (1944) in which it was indicated that bacteria present in low concentration on meat products exhibited lag periods of approximately 40 hours at 37°C.

d. The Tetrazolium Flooding Technique

In as much as the food particles were unable to reduce the indicator they remained their natural color and could not be mistaken for the now red bacterial colonies; thus, several of the errors which are normally present when plate counts of food products are extended into the low dilution range were eliminated.

The value of the Tetrazolium Flooding Technique in increasing the accuracy of the standard plate count in low dilutions of food products was unmistakeably demonstrated. The technique also resulted in the observation of small colonies which may go unobserved when ordinary procedures are employed. Colonies which are situated near the edges of plates which are badly scratched because of constant re-use very often go unobserved during plate counting. The TTC treatment made these colonies stand out, thus making them easily countable.

e. The simulated chicken model system

Since the experiments evaluating the nutritive contribution of various gelling agents to bacteriological media were of a screening nature, the sampling times selected were far apart. The information obtained confirmed the reports by Ryan (1952) and Miller (1959), both of whom observed considerable growth of microorganisms upon commerical bacteriological agar. The curve obtained for growth on the Purified agar indicated that Frobisher's (1944) definition concerning the inert qualities of agar were true, provided proper purification techniques were employed. E. coli, especially strain ATCC 9637, which is a wild strain, is able to reproduce in the presence of very few nutrients. The

organism is one of the most complex ones known and contains enzyme systems within it for the synthesis of almost every required nutrient provided it is supplied with an assimilable carbon source.

Bacto agar appears to contain impurities which are removable by repeated washing in water, alcohol and pyridine. Among these impurities are all of the essential nutrients which E. coli requires in order to reproduce. The Irish Moss extractive has available nutrients which are more readily utilized by E. coli or some auxiliary growth factors which increase the growth rate. There also seems to be more nutrient material present in the Irish Moss extractive than there is in the Bacto agar since the maximum number of organisms obtained by growth on this medium was greater.

The steady decline of the viable count upon the Purified agar is difficult to explain since the agar was not found to contain any inhibitory substances. An hypothesis which may explain the phenomenon follows. The protection offered by the broth which was transferred with the cells into the dilution blank may be lost by diffusion through the agar disc, away from the cells, thus leaving the cells sensitive to their environment.

The decreased adjustment phase period which was observed on the simulated chicken medium may be the result of the added moisture content in the system. Lichstein (1959) discussed the factors which determine growth of microorganisms which were seeded into new media. He listed the factors as: the suitability of the physical and chemical environment, the availability of the

medium components to the cell, and the adaptability of the cell. In the case in point, the physical and chemical environments were similar with the exception of the moisture contents and any effect which may have been the result of the heat treatment which was required in the preparation of the simulated medium. The adaptability of the cell was not a factor, since cells were the same to begin with. The availability of medium components could be considerably altered through the change in moisture content and the formation of the agar suspension. Since the duration of the adjustment phase on the heat treated whole chicken was the same as that on the non-heat treated whole chicken it was assumed that the heat treatment had no effect upon the adjustment period. The difference may have been a result of the change in physical form from a piece of whole chicken, in which diffusion rate of nutrients to the surface to replace those used by the microorganisms might be less rapid, to an agar suspension in which diffusion of nutrients could be more rapid. The water activity of the system would also be much greater in the agar gel system since the solids content, based on chicken breast muscle moisture content of 75% as reported by Morgan et al. (1949), would be reduced from 25% to approximately 7.5%. The increased water activity could, according to Christian and Scott (1953), raise the rate of growth throughout the growth curve but this did not occur, possibly because the high humidity storage chamber which served to raise the water activity at the surface of both media to the same equilibrium point was used. The establishment of a water activity equilibrium at the surface

was pointed out by Mossel and Ingram (1955). In their studies they found that foods stored in a non-sealed manner tended toward equilibrium in moisture with their surroundings, especially the surface of the food, and since, in the study being discussed, only the surface growth of the microorganisms was considered, the application of their findings appears valid.

The initially increased rate of growth which was evident on the simulated chicken medium as well as the heat treated whole chicken appears to be a result of the heat treatment. If an inhibitory substance was destroyed by the heating effect the curve would continue at its initial rate rather than assume a new slope which was very similar to the one which was obtained upon the raw untreated whole chicken. It is possible that the effect was the result of a release of nutritive material made possible by the mild heat treatment. Gale (1951) stated that very few bacteria were capable of breaking down protein to amino acids and E. coli was not among those few possessing this ability, but E. coli was capable of utilizing many amino acids for growth though it required only sources of nitrogen and carbon to manufacture its own amino acids.

The presence of more easily assimilated nutrients or nutrients which eliminate steps of synthesis will result in increased growth rates until some maximum growth rate is reached. It is possible that the mild heat treatment afforded the two media being discussed resulted in a mild hydrolysis of some sort with the resultant breakdown of complex materials into more simple and more easily utilizable forms. Since the nutrient release

would be small, due to the mildness of the heat treatment, the availability would last for only a short period, after which the system might be similar to the one which was unheated. The occurrence of multiple exponential phases of bacterial growth were discussed by Monod (1949) who stated that such observations should be interpreted as indicative of the addition or removal of one or more rate determining steps in the steady state system. Monod (1949) also cited reports of two specific examples of multiple exponential phases, which were explained on the basis of exhaustion of a compound partially covering an essential nutrient requirement and on the basis of the accumulation of a metabolite which eventually served as a secondary nutritional source.

Though this explanation appears logical and reasonable one must remember that there is a great deal unknown about the growth of microorganisms and the true explanation may be very different from the one projected here.

Due to the two differences exhibited by the simulated medium when compared to the raw whole chicken, its value to the thesis was questionable. Therefore, further experimental work with it was discontinued.

IV. THE GROWTH SUPPORT POTENTIAL OF VARIOUS SUBSTRATES FOR ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

A. General

With the knowledge that, for purposes of inoculating and counting, suspensions of the test organisms E. coli and Staph. aureus would survive through a 40 minute period; that each sample in a series could be inoculated with a uniform number of organisms which could subsequently be recovered and cultured to form colonies; that bacterial colonies could be distinguished from food particles; and that essentially sterile chicken meat could be obtained, the study of the effects of various processing techniques upon the growth support potential of the chicken meat for the test organisms could be undertaken.

It was first desirable to obtain information pertaining to the growth characteristics of the test organisms upon conventional bacteriological media which were nutritionally adequate, thus determining the growth response of the organisms under what may be termed ideal conditions.

Growth characteristics of the organisms upon chicken meat in the raw state and in the heat processed state were required in order to compare the responses observed upon the radiation processed products. The radiation dose levels chosen for study were in both the pasteurizing and the sterilizing range, as it is expected that products treated at both of these levels will eventually find their way into commerce.

Comparison between raw chicken, heat processed chicken, and radiation processed chicken at both pasteurizing and sterilizing doses, with reference to their growth support potential for E. coli and Staph. aureus were also desired in order to determine the relative effectiveness of E. coli as a sanitation index and of Staph. aureus as a potential health hazard after the various processes were effected. From this same data some information might be derived from which the safety of present day food handling techniques could be evaluated with respect to radiation treated foods.

B. Experimental Procedures

1. Maintenance of cultures and preparation of inoculum

Cultures of *Escherichia coli* ATCC 9637 and *Staphylococcus aureus* ATCC 9664 were maintained on Stock Culture (SC) agar (Difco) slants. The cultures were transferred every 15 to 20 days to duplicate SC agar slants which were incubated at 37°C. for 24 hours after which they were stored in a refrigerator at approximately 10°C. One of the two slants was used for subsequent culture transfer only while the second was used periodically to prepare experimental inocula.

An experimental inoculum of *E. coli* was prepared by removing cells from the stored slant by means of an inoculating needle and subculturing the organisms in a flask containing 100 milliliters of 37°C tempered Tryptone Glucose Extract (TGE) broth. The flask was incubated at 37°C for 18 to 24 hours after which another subculture was prepared by transfer of three loopfuls from the 18 to 24 hour culture to another flask containing 100 milliliters of tempered TGE broth. The newly inoculated flask was incubated at 37°C for 18 hours prior to use.

Subsequent transfers were made from broth to broth for a one week period, after which a transfer from the refrigerated slant was again prepared for use in the experiments of the next week.

An experimental inoculum of *Staph. aureus* was prepared in the same manner as that for *E. coli* except that Brain Heart Infusion (BHI) (Difco) was the nutrient medium and the cultures were allowed to incubate 24 to 30 hours instead of the 18 to 24 employed in the case of *E. coli*.

2. Preparation of substrate materials

a. Tryptone Glucose Extract Broth was prepared by dissolving 3.0 grams of Bacto-Beef extract (Difco), 5.0 grams of Bacto-Tryptone (Difco), and 1.0 grams of glucose in one liter of distilled water followed by apportionment into flasks and autoclaving at 121°C for 20 minutes.

b. Brain Heart Infusion agar (Difco), Brain Heart Infusion (Difco), and Tryptone Glucose Extract agar (Difco) were prepared in accordance with the instructions provided by the manufacturer. Discs of the agar media were prepared by use of the open end syringe method described previously in Section III, B, 1, b. Tubes, for resting culture studies, were prepared by adding 10 milliliters of BHI medium into pyrex glass test tubes, plugging the tubes with cotton and autoclaving the tubes at 121°C for 20 minutes.

c. Raw sterile chicken was obtained by the technique described previously in Section III, B, 1, d. Discs of raw chicken were prepared by use of the sterile cutting die method described in Section III, B, 1, f.

d. Heat processed chicken was prepared by placing raw sterile chicken meat in mylar (1.0 mil) bags which were sealed on a Vertrod thermal impulse sealer. Processing was accomplished in a Presto pressure cooker which was adapted for regulated air pressure cooling in accordance with a method described by Davis, Karel, and Proctor (1960). A process time of 60 minutes at 121°C was employed. After the cooling period the bags were opened and discs were cut using the sterile cutting die method (Section III, B, 1, f.).

e. Electron radiation processed chicken was prepared by trimming raw sterile chicken to uniform thickness as was described in Section III, B, 1, f., after which the plastic Petri dish covers containing the pieces of chicken were packaged in heat sterilized mylar (1.0 mil) bags which were then sealed on a Vertrod thermal impulse sealer. The packages were then iced and transported to the High Voltage Engineering Corporation in Burlington, Massachusetts. The packages were then placed on ice filled trays which were then placed on a moving belt which transported the samples below the beam of a 2 Mev (million electron volt) Van de Graaff electron accelerator. Each time a sample traversed the beam of electrons, the equivalent of one million rad (1 megarad) of ionizing radiations were absorbed at the surface of the sample. When the total dose required for an experiment necessitated more than two exposures of the samples to the beam, an extra precaution was taken to prevent excessive heating of the samples. Between each two exposures the samples were allowed to cool for a two minute period. After the irradiation was completed the samples were re-iced and returned to the laboratory in Cambridge, Massachusetts. Travelling time for the samples was approximately 40 minutes in each direction. Discs were then prepared from the irradiated chicken by use of the sterile cutting die method described in Section III, B, 1, f.

f. Gamma-radiation processed chicken was prepared in the following manner:

1. Raw sterile chicken meat was obtained and trimmed to uniform thickness by the methods described in Section III, B, 1, f.

2. Discs were prepared by the sterile cutting die method described in Section III, B, 1, f.

3. The discs were placed in two sterile pyrex glass tubes (approximately 25 discs in each tube) of the same diameter as the cutting die and both ends of the tubes were sealed with saran-covered rubber stoppers.

4. One of the tubes was placed in the Co^{60} facility and exposed to six megarad of ionizing radiations (165,000 rad per hour). The other tube was placed in the building housing the irradiator but out of the reach of any radiations.

5. The samples were removed from the tubes, placed in Petri dishes, and were now ready for inoculation or other treatment.

3. Determination of growth characteristics

a. Inoculation of liquid media or discs of solid media was accomplished through use of the inoculating syringe described in Section III, B, 1, a. Spreading of the drop over the surface of the solid substrate was accomplished with the T shaped rod by the method described in Section III, B, 1, b. The inoculum was usually adjusted to contain 3,000 to 30,000 organisms per drop, though a larger inoculum was employed in a few of the experiments. When E. coli was the test organism, inoculation was achieved by filling the syringe from a 1/100 dilution of the 18 hour culture propagated in TGE broth at 37°C. For Staph. aureus studies the syringe was filled from a 1/1000 dilution of the 24 hour culture grown in BHI at 37°C, with the following exceptions:

the early experiments involving growth on BHI agar, in BHI, and on raw sterile chicken were inoculated from a 1/100 dilution.

b. Incubation of liquid media took place in a constant temperature room at 37°C. Incubation of solid media samples took place within a high humidity chamber (described in Section III, C, 1, d) which was maintained in a 37°C constant temperature room.

c. Sampling from liquid media was accomplished by first thoroughly mixing the contents of the flask or tube in which the organisms were growing and then removing one milliliter aliquots from which the necessary decimal dilutions were made. Triplicate plate cultures were prepared from the dilutions.

Sample selection for solid media experiments was accomplished by estimating the time period for each of the phases of growth, determining the number of samples required to adequately study each phase, selecting this number of inoculated discs in order of inoculation, and then randomizing the discs prior to their selection as samples. At selected sampling times, every 30 or 60 minutes during the phase of adjustment, every 60 minutes during the logarithmic phase, and every 60 or 120 minutes during the stationary phase with some exceptions as may be noted in the data tables of Appendix I, duplicate discs (in one experiment triplicate discs) were chosen and removed from the incubator. One disc was placed in a sterile Oster blendor cup with modified blades (see Section III, B, 1, b). Ninety-nine milliliters of sterile chilled distilled water was added, the cover was placed on the cup, the cup and contents were placed upon a Waring blendor

base and were ground for four minutes. After 3 1/2 minutes of grinding, the second sample was transferred aseptically to a second sterile blendor cup and 99 milliliters of sterile chilled distilled water were added. The four minute blending period of the first sample was completed simultaneously with the readiness of the second sample for grinding. While the second sample was subjected to a four minute blending operation, decimal dilutions and triplicate plate cultures were prepared from the first sample. After completion of the four minute blending operation of the second sample decimal dilutions and triplicate plate counts were prepared from it also. All decimal dilutions were made in sterile chilled distilled water.

Zero time in all experiments was the time immediately after all samples were inoculated and had been placed in the incubator. The time lapse between irradiation and experimental zero time was kept approximately constant at two hours except in those experiments during which the effect of the time lapse was under investigation. In the time lapse studies, the samples were stored in a high humidity chamber at 37°C until they were to be inoculated.

d. The plate culture medium for all studies involving E. coli was TGE agar. The plate culture medium for all studies pertaining to Staph. aureus was BHI agar.

e. Incubation of the plate cultures took place at 37°C without humidity control, though occasional survey of the incubator humidity showed that it seldom rose above 20% R.H. (relative humidity) and was generally between 10% and 20% R.H. Plate cultures of E. coli were incubated 18 to 24 hours prior to counting

of the formed colonies while plate cultures of Staph. aureus were incubated 24 to 36 hours prior to counting.

f. Counting the number of colonies formed was accomplished through the use of a Quebec Colony Counter. Prior to counting the colonies on any plate culture prepared from the 1/100 or the 1/1000 dilutions, those plates were subjected to the Tetrazolium Flooding Technique described in Section III, B, 1, e.

4. Data analysis

a. Phase of adjustment data. The phase of adjustment was defined as the time required to double the initial number of viable organisms present in or on any sample.

Due to variations in the initial counts the adjustment phase data was analyzed on a per cent of initial count basis.

b. Logarithmic phase data. The data accumulated during the logarithmic phase of growth of E. coli and Staph. aureus on the various media investigated was subjected to regression analysis to determine the numerical value for the slope of the best straight line. Duplicate and triplicate slope results were grouped. The groups, each representing a different treatment, were then subjected to statistical analysis to determine the significance of observed differences. The 95% confidence limits about the slope values were determined. The slope values were also converted into generation time of the microorganism, expressed in minutes per generation.

Since the number of treatments was not very great, it seemed that the best method for determination of the significance

of observed differences was the subjection of the results to a series of t tests. The growth of the microorganisms upon liquid and solid bacteriological media were compared with one another. The growth on the various chicken samples were compared by arranging the results in all possible combinations of two, and then performing the analysis upon each pair.

Each pair being compared was first subjected to a test for homogeneity of variance by means of a variance ratio test at the 1% significance level. The pairs of slopes were then subjected to a t test and the significance as well as the degree of significance of the observed differences were determined.

c. Stationary phase data. The stationary phase was considered to be that period after the logarithmic phase when there was no regular increase or decrease in viable count.

The data are presented as the log of the number of organisms as determined by standard plate count methods.

C. Results

1. Phase of adjustment

Figures 4 and 5 represent the phase of adjustment for E. coli and Staph. aureus respectively, upon the various media indicated.

The differences between the various media, with respect to the growth response of E. coli, were small with the exception of the samples which were treated with six megarad of ionizing radiations. In this latter case the duration of the adjustment phase was noticeably increased and the decrease in viable count during the adjustment period was somewhat greater than that encountered on any of the other media studied.

The growth characteristics exhibited by the Staph. aureus organisms during the adjustment phase was somewhat different from those demonstrated by E. coli in that there was no marked decrease in viable count after inoculation on any of the media tested. The duration of the adjustment period varied in a manner similar to that observed in the E. coli experiments with all of the samples yielding similar results until the sterilizing doses of ionizing radiations were approached. On both the three megarad and the six megarad treated chicken samples there was a greatly increased adjustment time, the time on the six megarad treated samples being considerably greater than that upon the three megarad treated samples.

In order to compare the various media and their effect upon the duration of the phase of adjustment, an arbitrary point, exactly double the original number of organisms or when $N/N_o \times 100$

was equal to 200, was selected as the end of the phase of adjustment. Table 11 presents the duration of the adjustment phase in hours, as determined for each of the organisms studied.

Figure 6 presents a relationship which was observed between radiation dose and duration of the phase of adjustment for both E. coli and Staph. aureus. The log of the duration of the adjustment phase was apparently a function of the radiation dose.

A few preliminary experiments were performed in order to obtain a better understanding of the effect of ionizing radiations upon the adjustment phase of growth. Figure 7 illustrates the growth response of Staph. aureus on chicken which was exposed to six megarad of ionizing radiations from a Van de Graaff accelerator, incubated at 37°C for 14 hours, inoculated, and then incubated at 37°C. A comparison with the growth response of Staph. aureus upon similarly treated chicken but without the 14 hour time lapse is presented. The duration of the adjustment phase was significantly reduced when this organism was grown on the pre-incubated samples.

Table 12 presents data obtained when the time lapse between radiation exposure and inoculation was varied. The controls were unirradiated chicken which were treated exactly as the irradiated samples with the radiation step omitted. The number of Staph. aureus organisms per disc which appear in Table 12 were determined by sampling after an eight hour post-inoculation growth period. The data showed that the growth response on the irradiated samples became more nearly equal to that on the control

as the radiation to inoculation time lapse was increased.

The effect of dose rate upon the extension of the adjustment phase was studied by exposure of the chicken samples to gamma irradiation in a Co^{60} irradiation facility, the dose rate output of which was approximately 165,000 rad per hour. The six megarad was therefore accumulated in 36 to 37 hours as opposed to a few minutes required when the dose was administered by the electron accelerator. Figure 8 shows the phase of adjustment curve which was obtained from the Co^{60} irradiated samples. The control curve represents the data obtained from samples of chicken which were treated exactly the same as the test samples with the radiation treatment omitted. The dotted line in Figure 8 represents the growth rate of Staph. aureus on raw chicken which was inoculated without a 36 to 37 hour delay after slaughter. The data from which this curve was derived appears in Table 46.

A comparison of Figures 7 and 8 showed that the duration of the lag was identical at the two dose rates investigated.

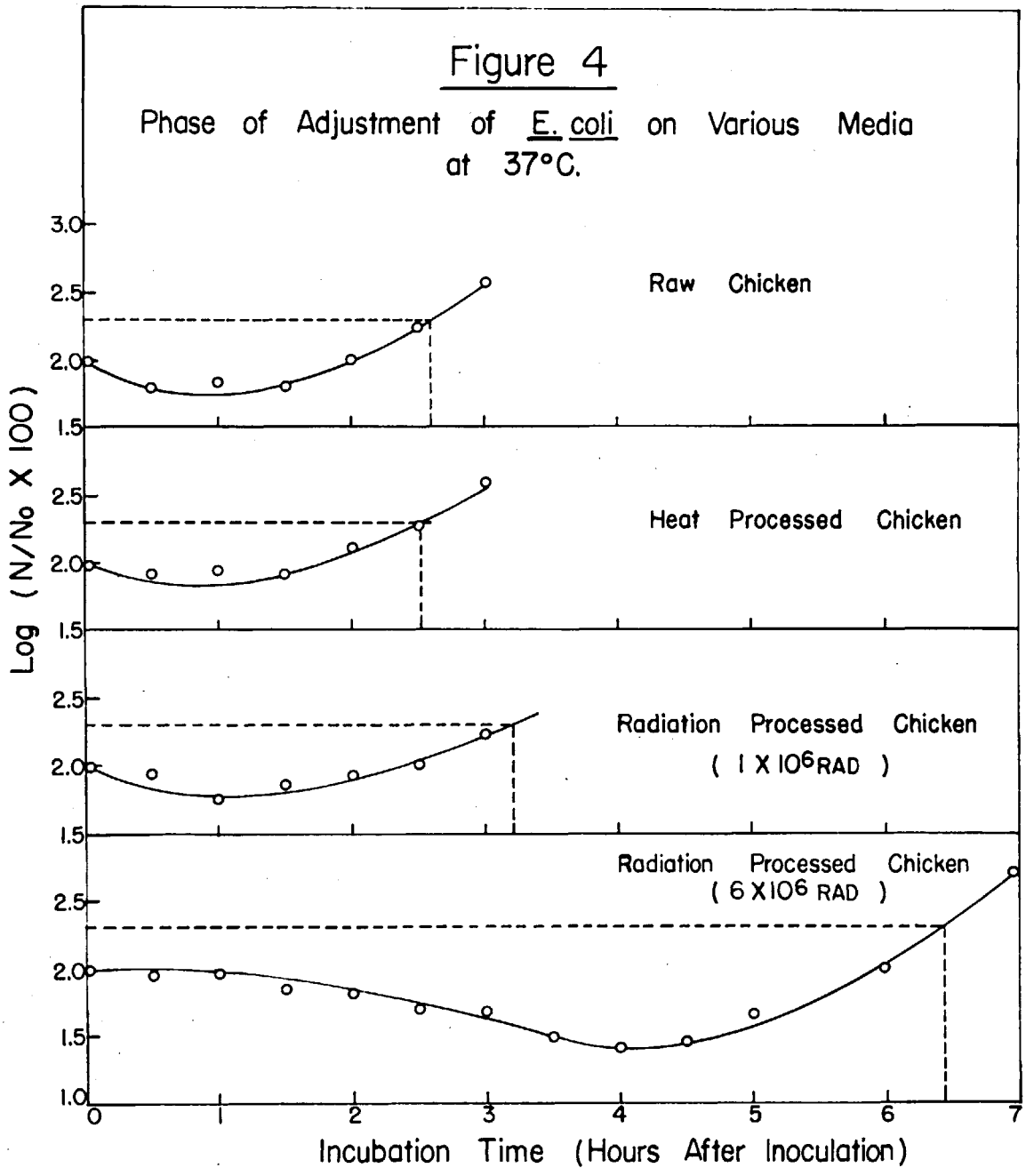


Figure 4
Phase of Adjustment of E. coli on Various Media at 37°C.
Raw Chicken
Heat Processed Chicken
Radiation Processed Chicken
(1 X 10⁶ RAD)
Radiation Processed Chicken
(6 X 10⁶ RAD)
Log (N/No X 100)
3.0
2.5
2.0
1.5
1.0
0
1
2
3
4
5
6
7
Incubation Time (Hours After Inoculation)

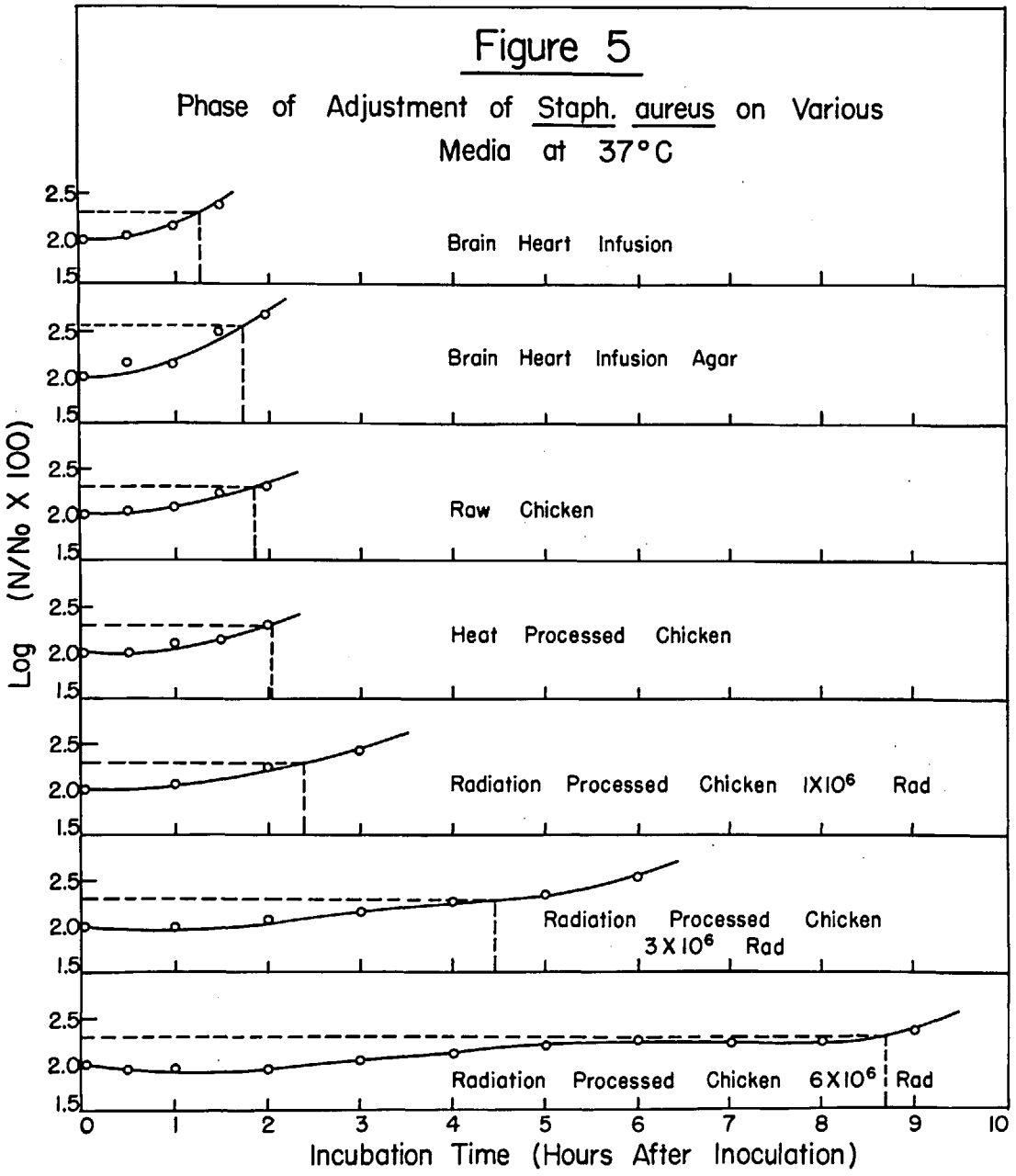


Figure 5
Phase of Adjustment of Staph. aureus on Various Media at 37°C
Brain Heart Infusion
Brain Heart Infusion Agar
Raw Chicken
Heat Processed Chicken
Radiation Processed Chicken 1X10⁶ Rad
Radiation Processed Chicken 3X10⁶ Rad
Radiation Processed Chicken 6X10⁶ Rad
Log (N/No X 100)
2.5
2.0
1.5
0 1 2 3 4 5 6 7 8 9 10
Incubation Time (Hours After Inoculation)

Table 11

The duration of the adjustment phase of E. coli and Staph. aureus upon various media at 37°C

Medium	Duration of adjustment phase (hours)	
	E. coli	Staph. aureus
Raw chicken	2.60	1.87
Heat processed chicken	2.50	2.05
Radiation processed chicken $1x10^6$ rad	3.20	2.40
Radiation processed chicken $3x10^6$ rad		4.47
Radiation processed chicken $6x10^6$ rad	6.45	8.70

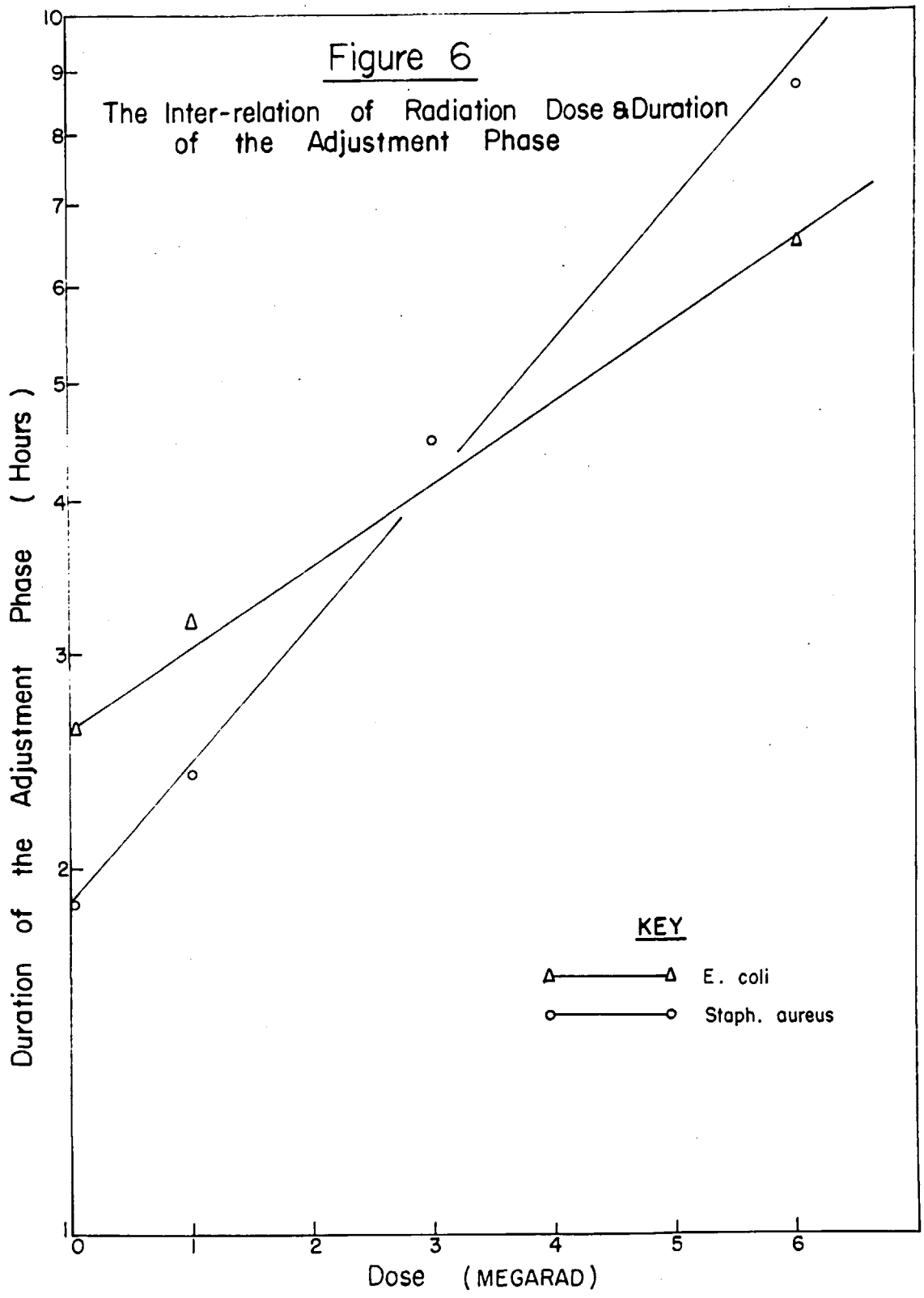


Figure 6
The Inter-relation of Radiation Dose & Duration of the Adjustment Phase
Duration of the Adjustment Phase (Hours)
Dose (MEGARAD)
KEY
E. coli
Staph. aureus

Figure 7

The Effect upon the Adjustment Phase of a 14 Hour Time Lapse Between Exposure to 6×10^6 Rad of Ionizing Radiations and Inoculation with Cells of Staph. aureus

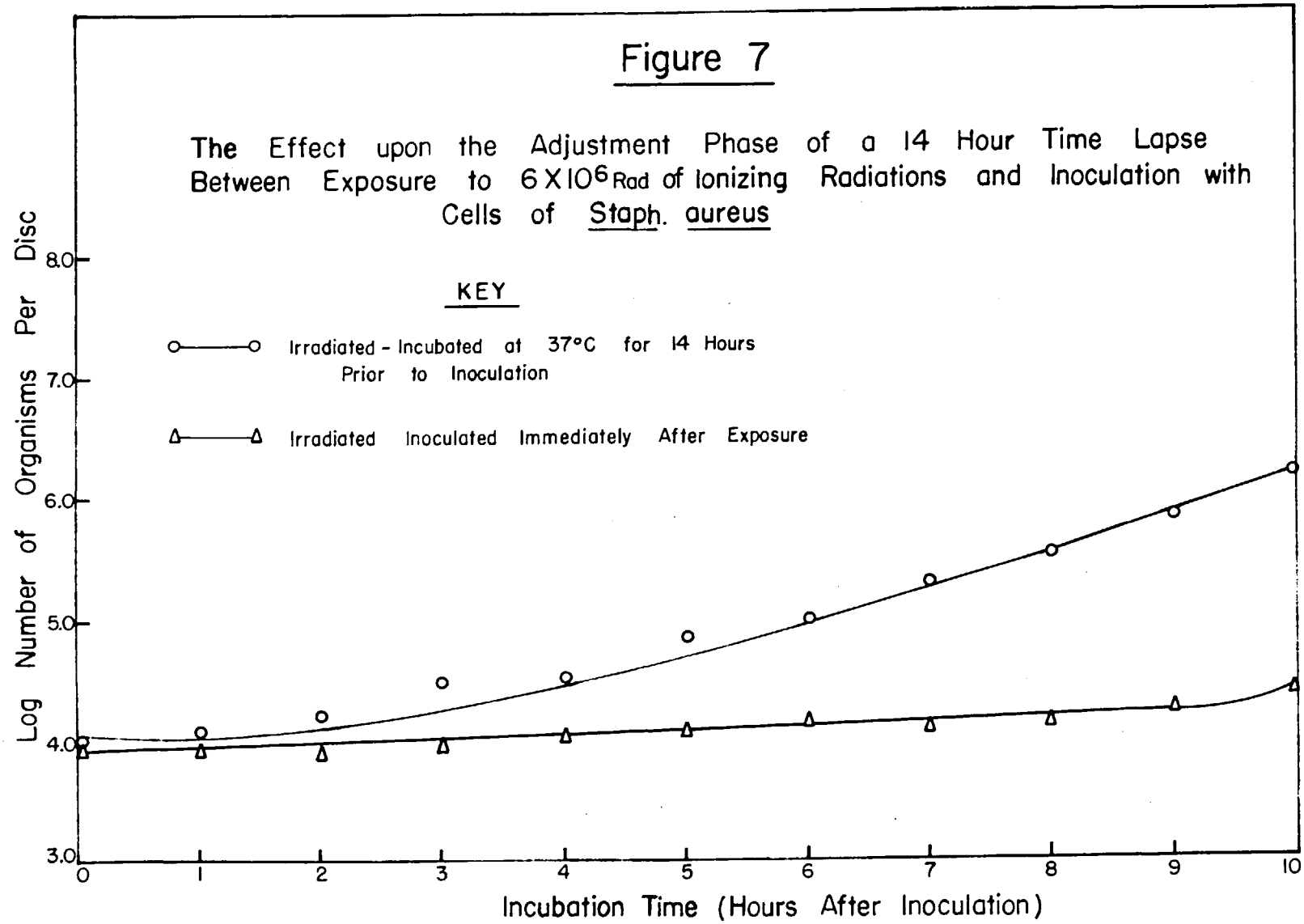


Table 12

The effect of various time lapses between irradiation* and inoculation upon the growth response of Staph. aureus on chicken meat treated with six megarad of ionizing radiations

Incubation time prior to inoculation	Organisms per disc 8 hours after inoculation		Irradiated Control
	Controls	Irradiated	
0	4.6×10^{7}	1.79×10^{4}	3.9×10^{-4}
5	1.07×10^{8}	4.0×10^{4} 6.9×10^{5}	3.7×10^{-4} 6.4×10^{-3}
14	2.00×10^{8}	6.3×10^{5}	3.2×10^{-3}
24	3.61×10^{8}	3.01×10^{8}	8.3×10^{-1}

*Ionizing radiations were obtained from a Co^{60} irradiation facility.

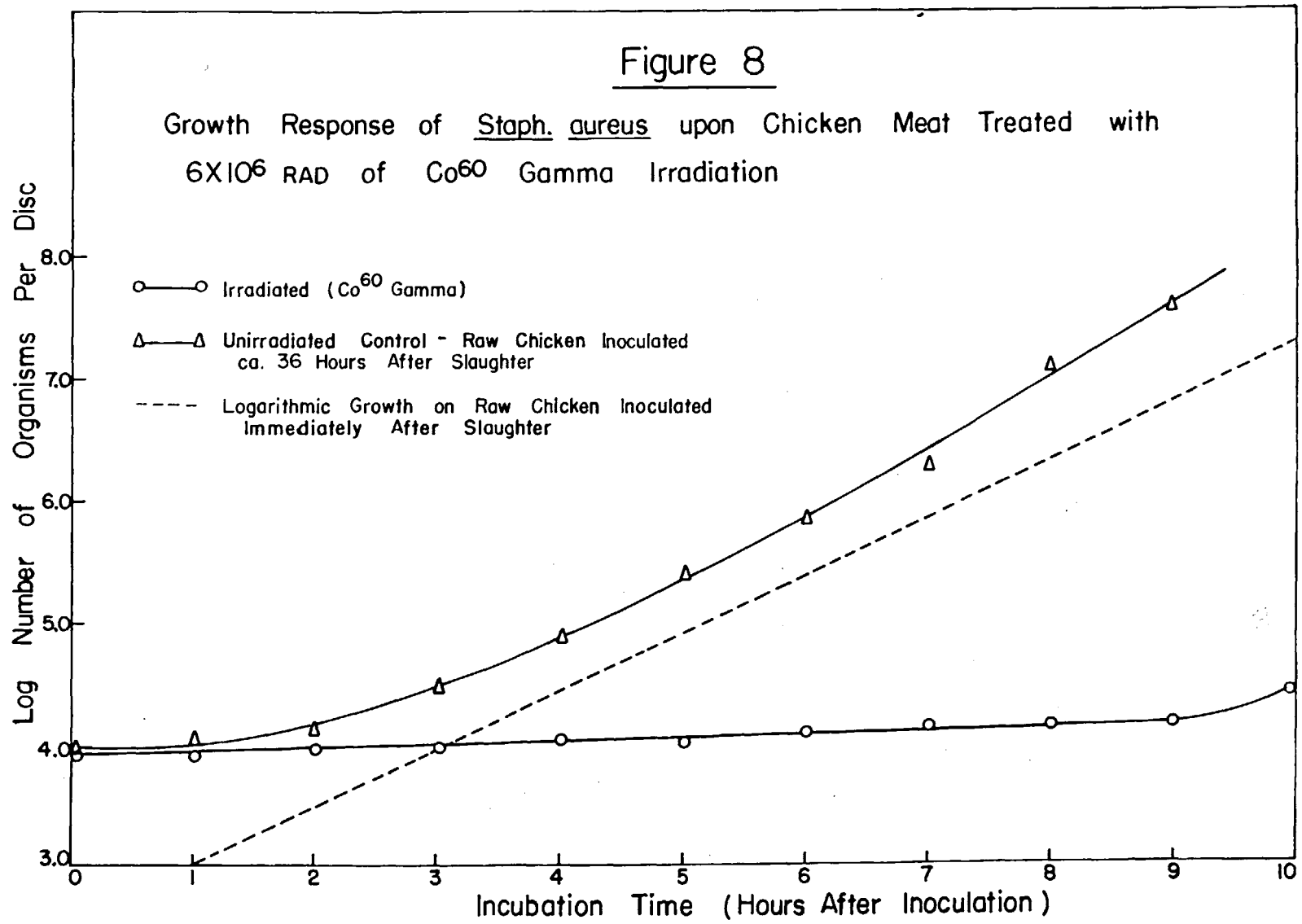


Figure 8
Growth Response of Staph. aureus upon Chicken Meat Treated with 6X10⁶ RAD of Co⁶⁰ Gamma Irradiation
Irradiated (Co⁶⁰ Gamma)
Unirradiated Control - Raw Chicken Inoculated ca. 36 Hours After Slaughter
Logarithmic Growth on Raw Chicken Inoculated Immediately After Slaughter
Log Number of Organisms Per Disc
8.0
7.0
6.0
5.0
4.0
3.0
0
1
2
3
4
5
6
7
8
9
10
Incubation Time (Hours After Inoculation)

2. Logarithmic phase

Figures 9 and 10 present the best straight lines, as determined by regression analysis of the data in Appendix IC and IF for the growth response of E. coli and Staph. aureus. on the various substrates indicated in the figures. The curves have been idealized and are all projected from a uniform initial point at which there is assumed to exist a single organism. This technique is equivalent to dividing all of the observed values by some initial value which is selected at a time after the organisms have entered the logarithmic phase of growth. In order to plot the experimental points of Appendix IC or IF and thereby observe their distribution about the regression line, one need only to subtract the value of $\bar{y} - b\bar{x}$, where $\bar{y}$ is the average of all of the observations of the log of the number of organisms, $\bar{x}$ is the average of all of the observations of time, and b is the slope as determined by regression analysis from the values for the log of the number of organisms in the Appendix tables.

The numerical values for the slopes together with their 95% confidence intervals are presented in Table 13. The mean generation times in minutes for both E. coli and Staph. aureus are also presented in Table 13.

All of the growth rates of E. coli and Staph. aureus on chicken meat, irrespective of the treatment to which the meat was subjected, were lower than those observed under the more ideal conditions offered by the TGE or BHI broth or agar.

E. coli exhibited a reduced generation time upon chicken meat after processing by either heat or ionizing radiations. The generation time of Staph. aureus on processed chicken meat was increased in every case except for the initial growth exhibited upon heat processed meat. The generation time upon heat processed chicken was less than that upon raw chicken for a period extending five to seven hours after inoculation or two to four hours after the organisms had entered into the logarithmic phase of growth. The secondary growth rate was logarithmic in nature as was the initial one, but the generation time was very much increased.

The slope values for the growth of each organism upon the variously treated chicken meat samples were arranged in all possible combinations of two for statistical analysis. A test of the variance ratio of each pair of slopes was performed at the 1% significance level and all differences were found insignificant with two exceptions, both of which occurred in studies pertaining to growth of Staph. aureus. The significant differences found were for regression data comparisons between samples treated with one megarad of ionizing radiations and samples which had been either heat processed or treated with six megarad of ionizing radiations. Each of the combinations of two slopes was then subjected to a t test to determine the significance of the observed differences between the slope values. The results of these tests are presented in Table 14 and 15.

Although all of the slope values representing the logarithmic phase of growth of E. coli on heat and radiation processed

chicken were greater than the slope value observed for the same organism on raw chicken; the logarithmic growth rate demonstrated on the chicken which was treated with six megarad of ionizing radiations was not statistically different from that demonstrated on the raw chicken even at the 5% level. The logarithmic growth rates of E. coli on chicken processed with heat or with one megarad of ionizing radiations were significantly greater than the growth rate exhibited upon the raw chicken at the 0.5% level. The differences in slopes obtained from the regression analyses of the logarithmic growth phase data for E. coli on all of the heat processed and radiation processed chicken samples were determined to be insignificant even at the 5% level.

In the logarithmic growth phase studies involving Staph. aureus the slope values exhibited on all of the processed chicken samples (heat, one megarad, three megarad, and six megarad) were lower than that which was observed upon the raw chicken meat with the exception of the initial or primary slope demonstrated by the organism when cultured upon the heat processed chicken. A comparison of the logarithmic phase slopes determined for Staph. aureus on any one of the chicken samples with that determined for the logarithmic growth response on any other of the chicken samples demonstrated a significant difference at the 0.5% level with the following exceptions:

a. Three megarad of ionizing radiations treated chicken vs. six megarad of ionizing radiations treated chicken, upon which there were no significant differences in the growth rate of Staph. aureus at the 5% level.

b. One megarad of ionizing radiations treated chicken vs. raw chicken, upon which there were no significant differences in the growth rate of Staph. aureus at the 5% level.

c. One megarad of ionizing radiations treated chicken vs. heat processed chicken (secondary slope), upon which there were no significant differences in the growth rate of Staph. aureus at the 5% level.

d. Three megarad of ionizing radiations treated chicken vs. heat processed chicken (secondary slope), upon which there was a significantly greater growth rate of Staph. aureus on the heat processed chicken at the 5% level but no significant differences at any of the higher levels.

A comparison between the logarithmic phase growth rates of E. coli on TGE agar and in TGE broth with hourly agitation showed no significant difference.

A comparison between the logarithmic growth rates of Staph. aureus on BHI agar and in BHI at rest showed that they were significantly different at the 0.5% level. Enough data was not obtained to statistically compare the BHI with periodic shaking to the BHI agar or to the BHI at rest, but there is evidence which indicates that the growth was the same as that upon agar although different from that in the resting broth.

Figure 9

Growth Curves for *E. coli* During the Logarithmic Growth Phase at 37°C

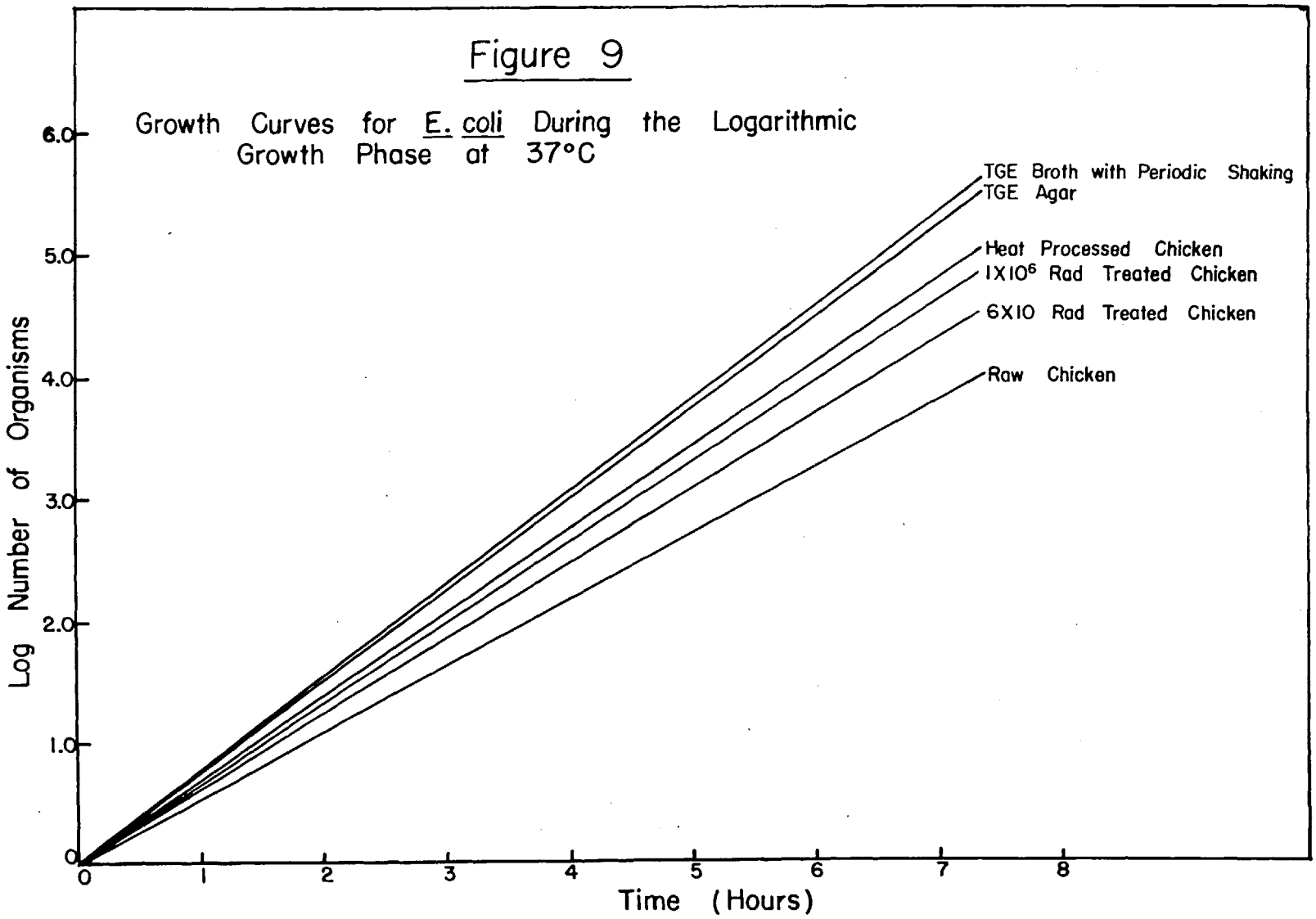


Figure 10

Growth Response Curves for Staph. aureus During the Logarithmic Growth Phase at 37° C

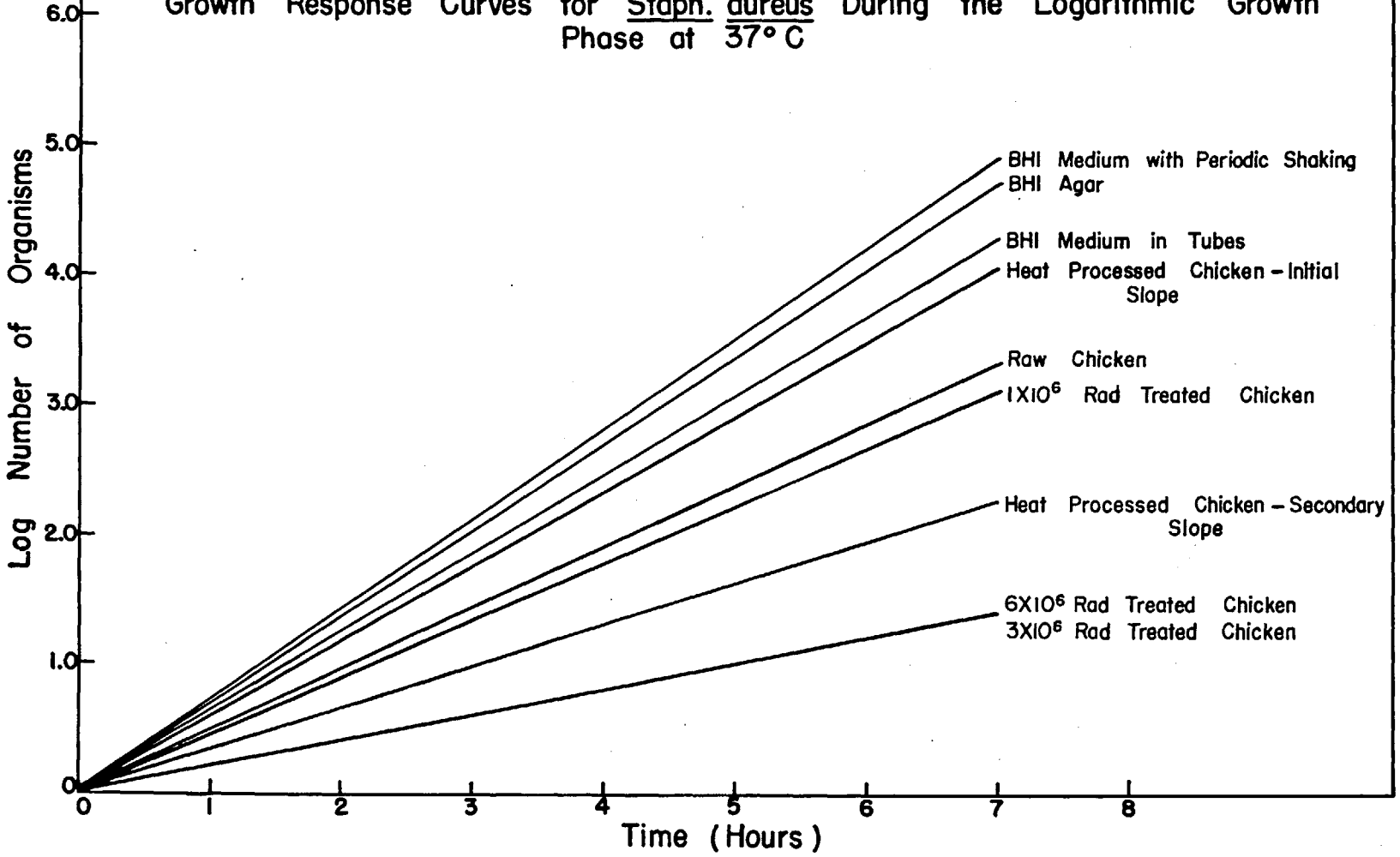


Table 13

Slopes of the logarithmic phase growth curves and generation times for E. coli and Staph. aureus on various media[1]

Medium	E. coli		Staph. aureus	
	Slope* ± 95% confidence interval	Generation time minutes	Slope* ± 95% confidence interval	Generation time minutes
Tryptone Glucose Extract Broth (agitated hourly)	0.7658±0.0307	23.58		
Tryptone Glucose Extract Agar	0.7427±0.0517	24.33		
Brain Heart Infusion(at rest)			0.6117±0.0247	29.52
Brain Heart Infusion Agar			0.6693±0.0152	26.98
Raw Chicken	0.5399±0.0591	33.45	0.4737±0.0360	38.13
Heat Processed Chicken	0.6883±0.0572	26.24	0.5801±0.0425[a] 0.3232±0.0896[b]	31.13 55.88
Radiation Processed chicken $1x10^6$ rad	0.6631±0.0515	27.24	0.4418±0.0456	40.88
Radiation Processed Chicken $3x10^6$ rad			0.2032±0.0297	88.88
Radiation Processed Chicken $6x10^6$ rad	0.6183±0.0632	29.21	0.1991±0.0028	90.71

[1] Data in Tables 25-30 and 43-51

* $\frac{d(\log \text{number of organisms})}{d(\text{time in hours})}$

[a] initial slope

[b] secondary slope

Table 14

t values obtained by comparison of slopes describing the response of E. coli during the logarithmic phase of growth on chicken meat subjected to the treatment indicated[1]

	Raw chicken	Heat processed chicken	Radiation processed chicken (1×10^6 rad)	Radiation processed chicken (6×10^6 rad)
Raw chicken		3.76*	3.19*	1.81
Heat processed chicken	3.76*		0.68	1.64
Radiation processed chicken (1×10^6 rad)	3.19*	0.68		1.03
Radiation processed chicken (6×10^6 rad)	1.81	1.64	1.03	

[1]Data in Tables 25-30

*Significant at 0.5% level.

Table 15

t values obtained by comparison of slopes describing the response of Staph. aureus during the logarithmic phase of growth on chicken meat subjected to the indicated treatments[1]

	Raw chicken	Heat processed chicken (initial slope)	Heat processed chicken (secondary slope)	Radiation processed chicken ($1x10^6$ rad)	Radiation processed chicken ($3x10^6$ rad)	Radiation processed chicken ($6x10^6$ rad)
Raw chicken		3.79*	3.49*	1.09	10.78*	14.96*
Heat processed chicken (initial slope)	3.79*		10.59*	3.68*	11.75*	15.00*
Heat processed chicken (secondary slope)	3.49*	10.59*		2.00	2.43**	3.13*
Radiation processed chicken ($1x10^6$ rad)	1.09	3.68*	2.00		8.50*	13.23*
Radiation processed chicken ($3x10^6$ rad)	10.78*	11.75*	2.43**	8.50*		0.26
Radiation processed chicken ($6x10^6$ rad)	14.96*	15.00*	3.13*	13.23*	0.26	

[1]Data in Tables 43-51

*Significant at 0.5% level

**Significant at 5% level

3. Stationary phase

Figure 11 presents a summary of the data which was obtained from studies of E. coli on various media during the stationary phase of the growth cycle. Each point in the figure represents the average of several samples varying in number from two to ten. The raw data is presented in Tables 31-35 of Appendix I.

A comparison of the curves shows that there is little difference between any of the media, though there may be a tendency toward a greater total cell density on the heat processed chicken and a lower total cell density on the raw chicken than there is on the other three media which are represented.

Figure 12 presents the data obtained during studies of the stationary phase of Staph. aureus in and upon various media. Each point represents the average of triplicate plate counts prepared from duplicate discs. Each change in symbols represents an experiment performed at a different time using media prepared at a different time. All of the data is presented in tabular form in Tables 52-58 of Appendix I.

The curves demonstrate rising and falling tendencies in many cases, as well as a tendency to rise as the incubation time becomes very long in the case of the chicken meat media. In the case of raw chicken, one run out of the five presented gave results which indicated a density of organisms somewhat below that which was normally present. This value rose into the normal range after a very long period of incubation.

When the earlier portions of all the curves are compared

with one another, some very great differences are evident. The maximum density of cells on Brain Heart Infusion agar is much higher than that upon or in any of the other media. All of the radiation treated samples are within the same general area of total cell density. Though there may be a trend indicated which would allow the placement of the one megarad treated samples slightly above the others. This is doubtful because of the large variations observed in the one megarad treated samples. The maximum density reached upon the heat processed samples is slightly greater than that upon any of the radiation processed samples, but the difference is not great enough to be considered important. The maximum number of organisms present per disc of raw meat appears significantly greater than that upon any of the radiation treated chicken samples, but there is some doubt of the significance of the difference observed between the raw meat and the heat processed meat due to the one raw meat sample on which the counts remained lower than on the other four samples of this type in maximum value attained during the early portion of the stationary phase.

When the very late portion of the curves are compared there seems to be a tendency for the cell density on all of the chicken media to approach a similar level. Although all of the chicken media exhibit an increase in total cells, there is a possible trend indicating that the ultimate height may be inversely proportional to the radiation dose.

The maximum cell density in BHI cannot be compared directly with that reached on BHI agar since the first is on a

milliliter basis while the latter is on a per disc surface (0.94 $in.^2$) basis. Since each disc contained approximately five cc. of medium a valid comparison may be made by multiplying the broth result by five and making the comparison on a per milliliter of medium basis. After this was accomplished the maximum cell density upon the agar medium was still approximately one-half of a log cycle above that in five milliliters of broth.

Table 16 presents the ranges of the maximum cell densities reached upon the various media expressed as log of the number of organisms per disc. This table serves to better define the differences and similarities discussed previously.

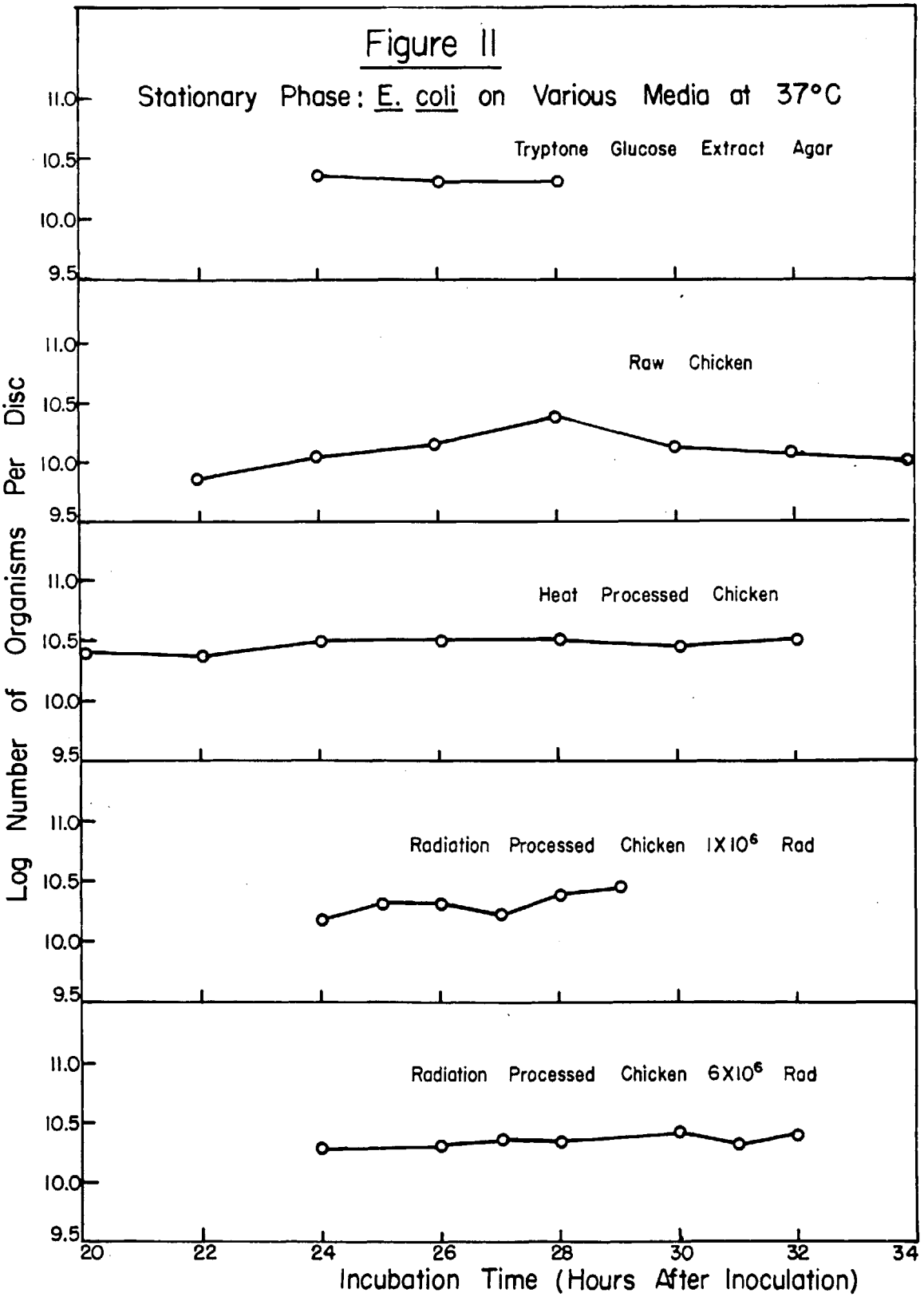


Figure II
Stationary Phase: E. coli on Various Media at 37°C
Tryptone Glucose Extract Agar
Raw Chicken
Heat Processed Chicken
Radiation Processed Chicken 1X10⁶ Rad
Radiation Processed Chicken 6X10⁶ Rad
Log Number of Organisms Per Disc
11.0
10.5
10.0
9.5
20
22
24
26
28
30
32
34
Incubation Time (Hours After Inoculation)

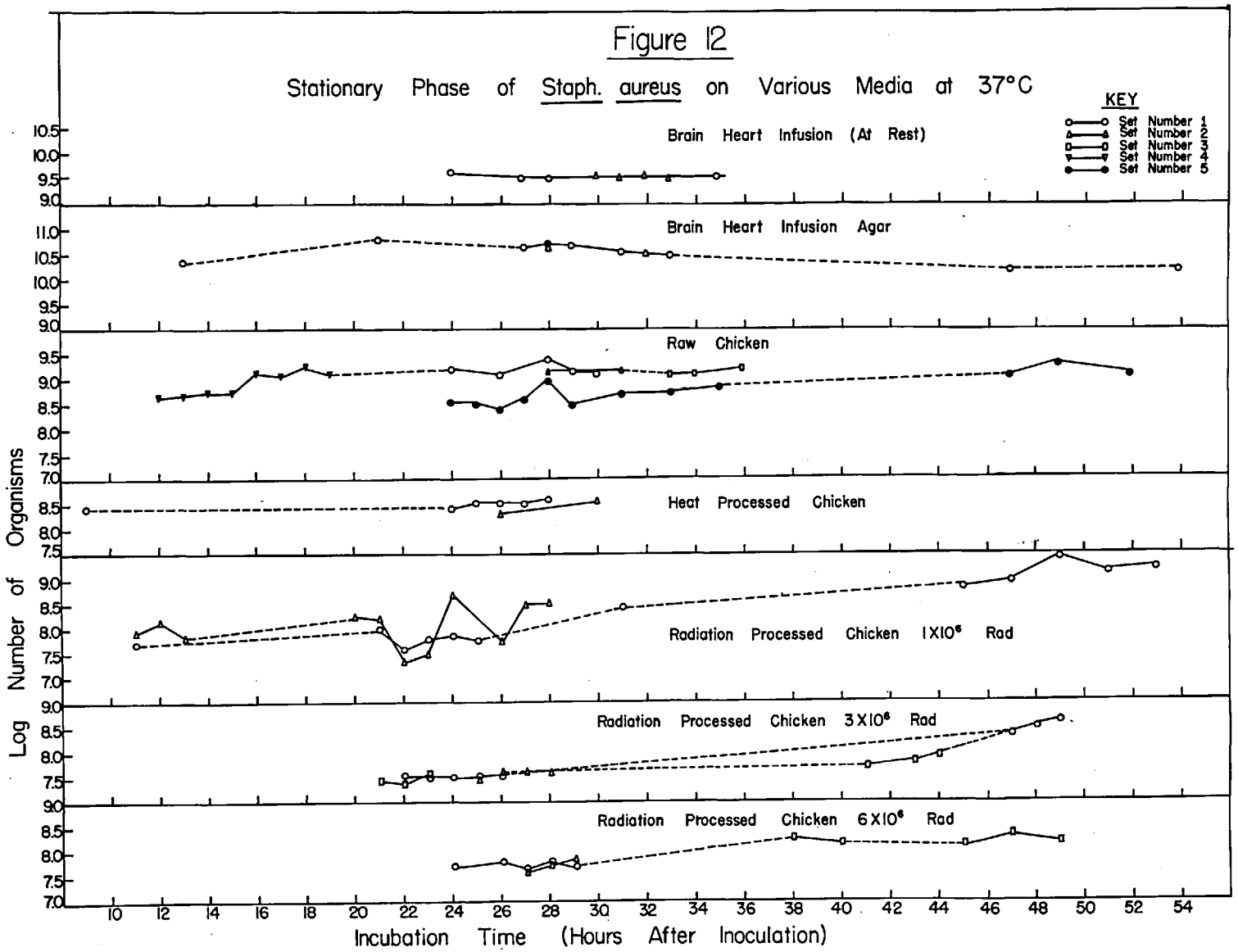


Figure 12
Stationary Phase of Staph. aureus on Various Media at 37°C
KEY
Set Number 1
Set Number 2
Set Number 3
Set Number 4
Set Number 5
Brain Heart Infusion (At Rest)
Brain Heart Infusion Agar
Raw Chicken
Heat Processed Chicken
Radiation Processed Chicken 1X10⁶ Rad
Radiation Processed Chicken 3X10⁶ Rad
Radiation Processed Chicken 6X10⁶ Rad
Log Number of Organisms
Incubation Time (Hours After Inoculation)

Table 16

Range of maximum cell densities per disc of media reached by Staph. aureus at 37°C

Medium	Range of Maximum Cell Density (log organisms/disc)	
	Early portion of stationary phase	Very late portion of stationary phase
Brain Heart Infusion Agar	10.4 - 10.8	10.1
Raw Chicken	8.5 - 9.5	9.0 - 9.3
Heat Processed Chicken	8.2 - 8.6	---
Radiation Processed Chicken (1×10^6 rads)	7.5 - 8.5	9.0 - 9.4
Radiation Processed Chicken (3×10^6 rads)	7.4 - 7.6	8.6
Radiation Processed Chicken (6×10^6 rads)	7.6 - 7.8	8.0 - 8.3

D. Discussion

This investigation was concerned primarily with the phase of adjustment (commonly referred to as the initial stationary and the lag phases), the phase of logarithmic growth, and the stationary phase of growth of E. coli and Staph. aureus on raw chicken, on heat processed chicken, and on radiation processed chicken; though some consideration was given to growth in and on common bacteriological media.

Most bacteriological growth studies which have been reported in the scientific literature have been carried out in liquid media. For the most part, the theories which have been proposed to explain bacteriological growth phenomena are based upon observation of events which took place in liquid media. Though Lamanna and Mallette (1953) hypothesized that no fundamental differences existed between bacterial growth in liquid media and on the surface of solid media, they immediately proceeded to describe a report in which maximum density of organisms per unit volume of environment was greater on solid medium than it was in liquid medium. This factor alone may cause the entire hypothesis to be suspect. The forthcoming discussion will consider microbial response upon solid media in respect to the theories and phenomena of liquid culture.

It is a generally accepted fact that in a liquid medium both the size of the inoculum and the nature of the new medium influence the duration of the adjustment phase. The larger the inoculum and the more similar the new medium is to that in which the organisms were previously propagated, the shorter will be the duration of the adjustment phase.

Evidence of a possible difference between the growth response of Staph. aureus in liquid medium (BHI) and on solid medium (BHI agar) was presented in the curves which described the adjustment phase. The adjustment phase appeared somewhat extended upon the solid medium. The tubes of BHI (10 ml. per tube) were inoculated with a number of organisms per milliliter equivalent to the number of organisms inoculated on the surface of five milliliters of solid substrate. Within the data in Tables 36 and 37 there may be seen ten-fold differences in the size of the inoculum which did not appear to influence the duration of the adjustment phase to any noticeable extent. Therefore, the five-fold differences in the size of the inoculum per milliliter of medium, which existed between the solid and liquid media can probably be neglected.

The nutritional content of both the solid and the liquid media were identical. The solid medium did contain an added 1.5% Bacto agar. From the data presented in Section III, pertaining to the growth support potential of Bacto agar, it would appear that the agar may serve to stimulate growth but should not inhibit or delay growth.

The similarity of the adjustment phase for both the BHI agar and the raw chicken, in spite of the very great differences in the nature of the media, may be construed as an indication that it is the physical nature of the medium which exerts the observed effect, since both the agar and the tissue may be considered as gels.

Some doubt is cast upon the difference when one considers that the broth tubes were pretempered to 37°C prior to inoculation while the agar discs were pretempered at room temperature. The influence of this temperature differential was not investigated.

The data collected during the growth studies of E. coli on bacteriological media (TGE broth and TGE agar) took place prior to the development of an interest in the adjustment phase. Therefore, the results obtained were not sufficient for comparison with those presented for Staph. aureus.

A comparison of the curves representing the adjustment phases of E. coli (Figure 4) and Staph. aureus (Figure 5) show they are quite different in one respect. The data for E. coli always showed a reduction in viable count after inoculation, whereas the Staph. aureus inoculum maintained its initial viable counts throughout the early portion of the adjustment phase. The decrease in viable cell count of E. coli appeared to increase in the radiation treated samples, but the data, which may be seen in Tables 23-24, was extremely variable with the result that no definite statement is possible. Preliminary data which was not included in this thesis indicated that the decrease did not occur upon transfer of the cells to fresh TGE broth. The possibility is thus presented that the loss of reproductive capacity of the cells of E. coli when inoculated upon the chicken meat, is a result of the drastic change in character of the medium. The sensitivity of E. coli ATCC 9637 (which was used in this study) toward transfer, demonstrated by loss of reproductive capacity of a portion of the total inoculum, was observed

by Brogle (1960), who utilized the same organism and performed growth curve studies in various liquid media.

The variations observed during the conduct of the experiments, especially during the period when the number of organisms was at the lowest point, were cause for concern, and required explanation. Unreported experiments of a screening nature were performed upon the effect of the chilled water dilution blanks used in the comminution operation. The results obtained confirmed those reported by Hegarty and Weeks (1940), that cold shock of E. coli during the logarithmic phase of growth resulted in destruction of as many as 90% of the cells present. The period just after the cells left the initial stationary phase and entered into the lag phase (phase of increasing growth rate) was the time when the change from zero sensitivity to 90% sensitivity occurred. It was during this period that the very large variations in results of the experiments being discussed seemed to occur. When the organisms reached the end of the adjustment phase the variations were somewhat diminished. Since the results were used for comparative purposes rather than as absolute values, the effect of the cold shock would not invalidate the results.

Staph. aureus organisms were unaffected or possibly very slightly affected by cold shock. The data obtained during the adjustment phase studies for these organisms displayed the expected variations for biological systems.

The increased length of the adjustment phase of E. coli when compared with Staph. aureus on raw chicken could almost

entirely be attributed to the cold shock phenomenon.

With Lichstein's (1959) statement in mind, that the most important factor in the determination of growth initiation is the completeness of the medium with respect to the nutritional demands of the microorganisms, we can proceed into a discussion of those responses of Staph. aureus and E. coli which were similar on the various chicken media.

The adjustment phases exhibited by the two organisms upon raw chicken meat and upon heat processed chicken meat were only very slightly different. The adjustment period tended to be slightly shorter upon the heat processed chicken than upon the raw chicken for E. coli, and visa versa for the Staph. aureus. A possible explanation for these differences will be presented later where a similar situation exists in the data of the logarithmic growth phase.

The adjustment phase duration was affected in a similar manner for both organisms when the chicken was treated with ionizing radiations. As the total dose administered to the samples increased, so did the duration of the adjustment phase. Since the effect was observed for both organisms, it suggested the possibility that it was not the result of the nutritional state of the medium because the strain of E. coli under study was a wild one which exhibits no requirement for complex nutrient factors, while the Staph. aureus organism has been shown to require thiamine, niacin, and free amino acids for growth. It may be possible to interpret the linearity displayed by both organisms when the log of the duration of the adjustment phase

was plotted against the total dose in rad as further evidence of the independence of the effect from nutritional factors. If the phenomenon was caused by the destruction of nutrients, we might expect to find a threshold level before which there was not enough destruction to hinder the development of the organisms. The curves obtained gave no evidence of an initial delay which again appears to suggest some effect of the radiation other than nutrient destruction. More evidence may be derived by looking ahead for a moment and considering some of the results obtained during the logarithmic phase of growth. All of the processed media supported the growth of E. coli equally, thus there was no indication of nutritional inadequacy. Chicken treated with both three megarad and six megarad of ionizing radiations supported the growth of Staph. aureus equally during the log phase as did raw chicken and chicken treated with one megarad of ionizing radiations. If we consider the log phase to be influenced by nutritional inadequacies, it would seem that there might be a critical region both at the beginning when there was not enough of a destructive effect and at the end, after the destructive effect became very great.

All of this evidence suggested the possible existence of an inhibitor which built up in concentration as the radiation dose was increased. The possibility that this inhibitor would lose its effectiveness as time elapsed after the completion of the radiation exposure seemed likely in view of a report by Pugsley et al. (1935) in which nutrient agar plates were exposed to large doses of X-rays and subsequently seeded with cells of

E. coli. Considerable death of cells was observed but it was further observed that as the time between the completion of the exposure and the inoculation increased, the amount of death decreased. These workers attributed the effect to the formation of a "toxic poison" which decomposed in a unimolecular manner.

Similar results to those obtained by Pugsley and his associates were obtained by Bedford (1927) but his radiations were ultraviolet rather than ionizing radiations. Another radiation after effect report was made by Stone et al. (1947). These investigators exposed sterile nutrient broth to ultraviolet radiations and found that when the broth was then seeded with Staph. aureus organisms there was as much as 100 fold increase in mutants produced over an unirradiated control broth.

The data presented in Figure 7 indicated that the observations made by Pugsley et al. (1935) upon agar exposed to X-rays were at least partially exhibited by chicken muscle exposed to ionizing radiations produced by an electron accelerator. The time lapse between irradiation and inoculation did result in a reduced inhibitory effect.

Since the inhibitory effect was found to dissipate as time elapsed the possibility that there would be significant dissipation of the inhibitory effect during exposure of a sample to a similar dose at a lower dose rate, thus resulting in a reduced inhibitory effect existed. The study of a significantly lower dose rate was reported in Figure 8. The results indicated that the accumulation of the inhibitor was independent of the dose rate within the range investigated.

The data in Table 12 was obtained from a single series of experiments and indicated that there was dissipation of the inhibitory effect whether the dose was from an electron accelerator or from a Co^{60} gamma irradiator, and whether the dose rate was very high (six megarad in a few minutes) or as low as 165,000 rad per hour (total dose of six megarad). Although the data was both incomplete and subject to greater than normal variations, a definite trend of growth approaching that of an unirradiated control was exhibited as the time lapse between exposure to ionizing energy and inoculation with Staph. aureus organisms was increased to 24 hours. The ratios of irradiated response to control response obtained during the experiment did not seem to fit a first order curve, but when this is considered it is evident the curve may well be of a higher order. If we assume that the decay of the inhibitory substance is first order then it is indicated that after the radiation ceases there is a reaction going on which may be described by the following equation:

$$C = C_o e^{-kt}$$

in which C is the concentration of inhibitor at time t, C_o is the concentration of inhibitor immediately after the exposure to radiations is completed, and k is the decay constant for the inhibitory substance.

After inoculation and after an adjustment period equivalent to that exhibited upon an unirradiated sample, it may be assumed that growth is occurring, or least attempting to occur in accordance with the following equation:

$$N = N_o e^{k't}$$

in which N is the number of organisms at time t, N_o is the number of organisms initially present, and k' is the growth rate constant.

The overall effect which would be demonstrated by a test sample should be a function of the sum of the two effects. This would be highly influenced by the inhibitor curve at first, but as that value decreased and the value of growth curve increased, the overall curve would be more and more influenced by the growth curve and would asymptotically approach it. The growth response demonstrated by the Staph. aureus organisms upon the irradiated chicken meat exhibited a considerable period of increasing multiplication rate which may be interpreted as the period during which the effect of the inhibitor was diminishing and becoming significantly smaller, thus allowing organism reproduction to influence the sum of the reactions to a greater extent. A similar effect may have occurred in the experiments involving E. coli, but because of the cold shock effect may not have been evident. The significantly shorter adjustment phase observed for E. coli when it was compared with Staph. aureus may be attributable to the shorter reproduction time which was demonstrated by the E. coli organisms (29.2 minutes vs. 90.7 minutes), thus causing the effect of the inhibitor to become insignificant while it was present at a somewhat greater concentration than it could have in the case of slower growing Staph. aureus organisms.

Though the data obtained was of a very preliminary nature and, as was pointed out previously, was incomplete and subject to unexplained variations, there may be a trend toward support of the proposed reaction mechanism. Since this study was not an integral part of the thesis, further investigations were not carried out.

During the exponential or logarithmic phase of growth nutrient availability, and accessory growth factors become important. Oginsky and Umbreit (1954) attributed the variations during the logarithmic phase of growth to penetration rate of nutrients through the cell walls, limiting concentrations of essential nutrients, presence or absence of accessory growth factors, and rate of conversion of nutrients to cell material. Here again, we must remember that almost all of the studies reported in the scientific literature were performed in liquid culture.

A difference was observed during logarithmic growth of a culture of Staph. aureus when the medium was changed from a liquid to a solid. The data presented in Figure 10 showed that the rate of growth was significantly greater on solid medium (BHI agar) than it was in liquid medium (BHI) at rest. There were indications that when the broth was periodically shaken, the difference in growth rate was eliminated. Though the data available for similar studies with E. coli was not as complete as that obtained for the Staph. aureus organisms, the difference in growth rates between aerated broth (TGE) and TGE agar appeared insignificant.

The difference observed was probably the result of limited oxygen supply to the unshaken or unaerated broth which was prepared as a deep tube culture. Since Staph. aureus is a facultative anaerobe the growth rates were probably not as different as those which might have been observed were an obligate aerobe the organism being studied.

The time period selected for logarithmic growth sampling on the radiation treated chicken appeared to be free of any influence of the radiation after effect which was so significant during the adjustment phase. It is fully possible, however, that the radiation effect may have been reduced to such a low level that it was not readily detectable among the numerous other sources of error which existed in the measuring techniques which were employed.

A comparison of the curves in Figure 9 (E. coli) and in Figure 10 (Staph. aureus)illustrates many differences which might possibly be explained on a basis of the extreme variation in the nutritional requirements of the two organisms. An attempt will be made in the next few paragraphs to propose possible mechanisms which might be responsible for the various curves which were observed.

Before any attempt is made to explain the observed phenomena, the role of the cold shock effect upon the logarithmic phase of growth must be presented. Staph. aureus organisms were unaffected by the exposure to cold water throughout their entire growth cycle. Unreported experiments conducted by the author indicated that throughout the logarithmic growth phase

of E. coli, exposure to chilled water in the manner which was employed in this work resulted in a constant per cent reduction of the viable bacterial count. Koch (1959) observed a similar effect in his work with E. coli and performed a series of experiments using radioactive tracers to measure the amount of cell lysis throughout the growth cycle. He found that during the logarithmic phase there was a constant amount of radioactive material released into his substrate, thus indicating that a constant fraction of the cells were susceptible to the treatment. These results substantiated those obtained by the author and add further credence to the opinion that the relative growth characteristics will be unaffected even though the absolute effects were invalid.

The increased growth rate demonstrated by E. coli upon all of the processed media, when comparison was made with the raw chicken, may be a manifestation of the hydrolysis or breakdown which occurred during the processing operation. The organism involved was a wild strain which required only a carbon source, a nitrogen source, and some minerals for growth, as was shown by Brogle (1960) who propagated the same organism in a minimal broth composed of glucose, ammonium sulfate, and sodium, potassium, and magnesium salts. In view of this, the increased growth rate may have been caused by the presence of more easily assimilated nutrients or stimulatory factors.

Drake and Giffee (1957) reported the partial hydrolysis of protein by the action of ionizing radiations. The hydrolysis of protein by heat is a well known phenomenon. Morgan and Siu

(1957) reported the occurrence of higher metabolic energy values in irradiated diets and stated that it was probably due to greater digestibility of protein and carbohydrate in the diet. Mandelstam (1958) and Imamurax (1957) reported increased growth rates in E. coli cultures when certain amino acids were present. All of these reports appear to indicate that both the radiation and heat could result in more readily available nutrients and that these nutrients could serve to increase the rate of growth of E. coli.

The logarithmic phase growth curves for the more fastidious Staph. aureus upon the various chicken media were very different from the ones observed for E. coli. The differences in growth response of Staph. aureus upon chicken subjected to different treatments appears to fit explanation on a destruction of nutrients basis, as was mentioned earlier during the discussion of the radiation after-effect.

Staph. aureus displayed a greater growth rate in its initial logarithmic phase upon heat processed chicken than it did upon any other chicken medium. All of the other growth rates were below that which was achieved upon the untreated chicken, including the secondary logarithmic phase upon the heat processed chicken. The growth rates on raw chicken and on chicken treated with one megarad of ionizing radiations were not statistically different.

According to Breed et al. (1957), Staph. aureus requires thiamine, niacin, and free amino acids for growth. Morgan and Siu (1957) stated that thiamine was readily destroyed by ionizing

radiation treatment of food products with as much as 70% destruction at one megarep and greater destruction at higher dose levels being more the rule than the exception. Niacin was reported much more resistant to radiation than thiamine, but there was measurable destruction.

It was stated earlier in this discussion that growth of microorganisms depends upon cell wall penetration by nutrients. Lockhart and Powelson (1954) offered a theory which, in essence, stated that the number of absorption sites upon the surface of an organism whichwere filled by a nutrient determined the cell wall penetration rate of that nutrient. When nutrient material is present at a concentration greater than some critical concentration, the absorption sites are readily filled and growth progresses at a rapid and uniform rate. As the concentration of an essential nutrient approaches the critical concentration, more time is required for the absorption sites to become filled, the penetration of the nutrient into the cell decreases, and the reproduction rate of the culture decreases. When the nutrient concentration reaches the critical level all growth should cease unless there is available a substitute nutrient which will replace the one which has become critical. When such a substitute is available it is generally of such nature that its penetration rate or its utilization rate is slower than the preferred nutrient and growth will progress at a reduced rate. Evidence of such a system was discussed by Monod (1949).

It may be possible to relate some of the logarithmic phase growth phenomena demonstrated by Staph. aureus on chicken

meat with some of the facts concerning the nutritional requirements of the organism previously presented, the destructive effects of processing, and the release of nutrients by processing. It has already been pointed out that both heat and radiation processing could release amino acids and could destroy thiamine. Drake and Giffee (1957) stated that radiation treatment of proteins can result in aggregation or polymerization as well as fragmentation or release of amino acids. It appears possible to interpret the logarithmic growth phase curves of Staph. aureus on the differently treated chicken samples in light of the possible protein fragmentation and aggregation, thiamine destruction, and the many other reported nutritional effects of radiation treatment on various pure and biological systems.

Before entering into the discussion of the stationary phase on the chicken media, it should be pointed out that again during this phase there was observed a difference between the growth response of Staph. aureus in broth and agar (Figure 12). Even after correction of the data to equalize the amount of medium available for the support of growth, the difference persisted. This confirmed the report of Lamanna and Mallette (1953) which was mentioned earlier.

A discussion of the theories of the stationary phase would be superfluous here since there are discussions in many text books of bacteriology and none of the proposed theories has, as yet, been fully accepted as the explanation. Wilson and Miles (1955) presented a brief but informative summary of the theories. There were two publications which described

work on solid media systems and therefore should be mentioned. Wynne and Norman (1953) reported the levelling of the growth curve and attributed it to the presence of inhibitory substances. Levine (1953) studied the staling of membrane filter cultures and reported it due to exhaustion of nutrients and not direct antagonism. These two reports alone demonstrate that the controversies in the scientific literature concerning the stationary phase are many and remain unsolved.

The observations made during the stationary phase of growth correlated well with those observed during the logarithmic phase of growth for both organisms on all of the test media. The general results may be expressed thus; the higher the growth rate during the logarithmic growth phase, the higher was the maximum cell concentration achieved during the early part of the stationary phase of growth.

The data in Figure 11 was presented on an individual run basis due to the observed oscillating effect. The variations are not understood though there are several possible explanations. Autolysis of the substrate materials may be eliminated as a possibility since the trend was evident on the heat processed samples in which enzyme action could be considered absent. Lysis of cells with release of nutrients may explain the phenomenon since this would account for both the rising and falling aspects of the effect. A mutant cell theory, proposed by Topley and Wilson (1946) as an explanation for a rising and falling effect observed during the phase of decline of a Salmonella specie may also serve to explain the observed phenomenon.

The effect might well be attributed, at least in part, to the experimental errors which may be an inherent part of the experiment. Biological variations or even slight variations in sample size and sample treatment could result in a difference of one or two generations in a pair of similar samples during the stationary phase of growth.

As the stationary phase studies were extended in time for the Staph. aureus organisms, there was a definite trend upward for the maximum cell density in all of the samples observed. Since no data was collected for the very late stationary phase on heat processed chicken, little more can be stated concerning the cause of the maximum cell density increase.

The following discussion will attempt to present some of the more practical aspects of the observed growth responses of E. coli and Staph. aureus upon the test media.

There are no federal standards in the United States pertaining to the allowable numbers of coliform organisms in food products with the exception of shellfish, at the present time, but the coliform concentration is regarded by many as a measure of cleanliness or sanitation in food processing. The Commonwealth of Massachusetts (1959) established a tentative standard of not more than ten coliform organisms per gram of frozen food which will become effective in August, 1960. Thatcher (1955, 1958) presented the case for the establishment of microbiological standards for foods but pointed out the need to treat many products on an individual basis in order to establish realistic standards, because of the indigenous microbial

populations associated with many food products.

Some food processors and some state public health departments have considered the adaptation of the milk standards to food products. Such an approach has been unsuccessful. Since the pasteurization process is sufficient to destroy all of the coliform organisms in milk, except in a case where the contamination may be extremely great, the milk industry generally considers coliform organisms as indicative of post-treatment contamination. The same significance could probably be assigned to coliform organisms in pasteurized and sterilized food products.

The data presented in Figure 4, 9, and 11 indicated that the growth response of E. coli upon chicken meat, whether the meat was heat processed, radiation pasteurized, or radiation sterilized, was similar after the adjustment phase and would result in slightly higher total numbers per unit time than would be obtained upon raw meat if conditions were such that growth was encouraged. The major difference which was observed was the extension of the duration of the adjustment phase. This was found to be influenced by a radiation after-effect which seemed to dissipate as time after irradiation exposure elapsed. A similar effect was observed for the Staph. aureus organisms which suggests the possibility that many, if not all, microorganisms may be similarly affected.

Since the loss in viable cells during the adjustment phase was not significantly different on any of the media, and since much of the observed loss of E. coli may have been a result of the cold shock effect, it seems quite possible that any post-

treatment contamination would be equally recoverable from all of the media irrespective of the treatment to which it may have been subjected.

If, when the radiation after-effect is further investigated, it proves to be effective against a wide variety of microorganisms, the validity of the coliform index will be better established. At present, it can only be stated that it appears possible to assign equal significance to the coliform concentration in foods processed by ionzing radiations as is assigned to the coliform density in foods handled by today's commercial methods.

The data presented in Figures 5, 10, and 12 demonstrate a decreased, or at the maximum, an equal growth support potential of irradiated chicken meat for Staph. aureus organisms. This may indicate that irradiation treated foods are equally likely or possibly even less likely to cause Staphylococcal food poisoning than are the now commercial products of a similar nature. Dack (1956) pointed out the fact that Staphylococcus enterotoxin production was directly related to the growth of the microorganism producing it and large numbers of organisms were required to be present before a product could result in a toxic reaction under normal circumstances. Since the growth rate appeared, in most cases, to be limited after the radiation treatment, a greater extent of time would probably be required for the production of a significant amount of toxin, thus allowing more handling leeway or adding a possible safety factor to the methods in use at the present time.

Little importance can be assigned to the increased adjustment phase period in this portion of the discussion, since the time lapse between the radiation treatment and the recontamination of the product will very likely exceed the 10 to 24 hour period during which the effect was noticeable. It is possible that the radiation after-effect may be prolonged by low temperature storage. Such an effect would probably add additional hours to the safe handling time if the product were recontaminated immediately after the processing was completed. If the treatment was one of pasteurization alone, the lag of the new contaminant or of the surviving organisms might be significantly increased, thus adding some time to the shelf life of the product.

V. SUMMARY AND CONCLUSIONS

The objectives of this thesis were to determine the effects of ionizing radiations on the growth support potential of chicken meat for Escherichia coli and Staphylococcus aureus, two microorganisms of public health significance. The investigation was divided into two major categories: (1) the development and evaluation of methods for measuring growth response of the microorganisms, and (2) the growth response of the microorganisms on bacteriological media, raw chicken meat, heat processed chicken meat, radiation pasteurized chicken meat, and radiation sterilized chicken meat. The growth response studies were sub-divided into three categories: (1) the phase of adjustment, (2) the logarithmic phase, and (3) the stationary phase.

A. Development and evaluation of methods

Twenty-four hour and 18 hour cultures of Staph. aureus and E. coli, respectively, maintained full viability when suspended in sterile chilled distilled water for periods as long as 40 minutes at concentrations of 10^5 to 10^6 organisms per milliliter.

Equal numbers of colonies were obtained in plate cultures prepared from drops of organism suspensions which served as inoculum for sterile chilled distilled water blanks and for discs of agar medium. The organisms which were placed on solid substrate discs were prepared for plate culturing by comminution with sterile chilled distilled water in a mechanical blendor cup.

Sterile chicken muscle was obtained for use in experimental work by employment of surgical techniques for preparation of the carcass, operation within an enclosed chamber under ultraviolet light, and use of aseptic technique during the excision of the chicken muscle. The pectoralis major muscle was used in all of the studies.

Chicken meat samples of reasonably uniform thickness were obtained through a slicing operation using the lip of a small plastic Petri dish as a thickness guide. Uniform surface area of sample discs was achieved through the use of a sterile brass cutting die.

All samples within any one experiment were inoculated with an equal number of organisms by the delivery of a uniform drop of inoculum to the surface of each sample. This was accomplished by placing a syringe and hypodermic needle filled with inoculum into a micrometer type device. By the turning of a screw the syringe plunger was then driven forward at a rate which permitted the formation and release of uniform size drops. The drops were spread over the surface of the substrate medium with a sterile glass rod.

A technique for recognition of bacterial colonies was developed, making use of the ability of microorganisms to reduce the colorless 2,3,5 triphenyltetrazolium chloride to the red formazan form. This resulted in readily identifiable microbial colonies in plate cultures even when large quantities of chicken tissue were present. The technique consisted of a post-incubation

flooding operation which obviated the inhibitory effects of 2,3,5 triphenyltetrazolium chloride to bacterial growth.

All of the above listed methods and techniques were evaluated by routine comparison tests and were found to be satisfactory for the measurement of the growth response of microorganisms on solid substrate.

B. The growth response of microorganisms on various media

1. Brain Heart Infusion medium (BHI) and Brain Heart Infusion agar (BHI agar)

a. Comparative results were obtained for the growth responses of Staph. aureus in liquid medium (BHI) and on solid medium (BHI agar) during the adjustment, logarithmic, and stationary phases of the growth cycle.

During the adjustment phase the growth of Staph. aureus appeared to be delayed on the solid medium.

During the logarithmic phase there was an accelerated growth rate of Staph. aureus on the solid medium as compared to growth in liquid medium at rest. This difference was eliminated by periodic shaking of the liquid culture.

Since the difference was eliminated by periodic shaking of the liquid culture, it appeared that the difference for aerobic bacteria may be attributed to the variation in oxygen tension which exists between a broth culture at rest and a culture growing in either aerated broth or on the surface of agar media.

During the stationary phase a greater cell density of Staph. aureus organisms per milliliter of available substrate was observed on the solid medium when comparison was made with liquid culture.

b. The results and conclusions presented above showed that differences exist in the growth support potential of nutritionally equivalent media depending upon whether the media are in the liquid or solid state. The differences observed did not appear to be of great magnitude.

2. Chicken meat media

a. The growth support potential of raw chicken meat, heat processed chicken meat, radiation pasteurized chicken meat, and radiation sterilized chicken meat for Staph. aureus and E. coli was investigated during the adjustment, logarithmic, and stationary phases of the growth cycle.

During the adjustment phase an effect inhibiting the growth of E. coli and Staph. aureus was observed on chicken meat which was exposed to total doses of one, three, and six megarad of ionizing radiations. This effect resulted in an extension of the duration of the adjustment phase for both microorganisms.

The inhibitory effect increased in extent as the total dose of ionizing radiations applied to the chicken meat increased, regardless of the rate at which the dose was applied. The quantity of inhibitor formed seemed to be a function of the total dose of ionizing radiations administered to the chicken meat.

The inhibitory effect diminished as time between irradiation exposure and inoculation of the chicken meat with microorganisms increased.

Assuming the difference in growth rate was caused by the presence of an inhibitor it would appear, in view of the dose rate independence of the inhibitor build up, that the inhibitor was continually produced during the exposure of the chicken meat to ionizing radiations at a rate which was proportional to the rate of input of ionizing energy.

There was little difference between the duration of the adjustment phases of Staph. aureus and E. coli on either raw or heat processed chicken meat.

During the logarithmic phase of the growth cycle on chicken meat, E. coli organisms appeared to be less dependent upon nutritional factors than did Staph. aureus. E. coli demonstrated a greater multiplication rate on both heat and radiation processed chicken meat than on raw chicken meat.

When the reproduction rate of Staph. aureus on heat or radiation processed chicken meat was compared with that on raw chicken meat it was found to be reduced in every case except for the initial response observed on the heat processed chicken meat.

The contrasting effects of the processing which were evident when the log phase growth of E. coli was compared with the log phase growth of Staph. aureus on chicken meat appear to be related to the differences in the nutritional requirements of the wild strain of E. coli, which will grow when supplied with a minimum of nutrients, and the more fastidious Staph. aureus which must have a considerably more complex group of nutrients to establish growth, some of which are subject to destruction by heat and ionizing radiations.

During the stationary phase of the growth cycle on chicken meat, both the Staph. aureus and the E. coli reached maximum bacterial densities which appeared to be related to the growth rates demonstrated during the logarithmic phase of growth. The trend indicated was: the greater the growth rate during the logarithmic growth phase, the greater the final maximum density of microorganisms on the chicken meat.

b. Based on the observed results, it is probable that the use of the coliform concentration as an index of the sanitary quality is just as valid for irradiated foods as it is for raw or heat processed foods.

Consideration of the radiation produced inhibitory after-effect and the rates of growth of the microorganisms during the phase of logarithmic growth indicates that should radiation processed chicken meat (dose levels greater than one megarad) become contaminated by Staph. aureus or E. coli organisms, immediately after processing, and should conditions be favorable for growth of these organisms, the time required for the production of a significant population density would be considerably longer than the time which would be required to reach the same population density if the contamination occurred on either raw or heat processed chicken meat. The time required for the development of a significant population density of contaminating organisms under the conditions stated above would increase as the total radiation dose increased up to six megarad, which was the highest level tested.

These observations may be considered as indicative of a safety factor which would be introduced in the handling of irradiation processed foods.

If the contamination of the chicken meat were not to occur until a considerable period of time had elapsed after completion of the radiation exposure (five hours for E. coli, ten hours for Staph. aureus), the time period required for the production of a significant number of E. coli organisms on the irradiated chicken would be approximately equal to that required on heat processed chicken and slightly less than that required on raw chicken. The time required for the production of a dangerous population density of Staph. aureus organisms in the radiation treated chicken meat would still be greater or at least equal to that required on the heat processed or raw chicken. This is due to the fact that the multiplication rate of Staph. aureus is significantly reduced upon chicken meat which has been treated with three or six megarad of ionizing radiations and slightly reduced on chicken meat which has been treated with one megarad of ionizing radiations, regardless of the time lapse between irradiation and contamination of the meat.

These observations indicate that radiation treatment of some food products may result in making that food product a less suitable culture medium for potentially dangerous microorganisms. This could be considered as the introduction of a safety factor to the distribution and marketing of the product concerned. Such would appear to be the case when chicken meat is considered in relation to staphylococcal food poisoning.

VI. SUGGESTIONS FOR FUTHER RESEARCH

An investigation of the type reported in this thesis opens many avenues of interest for further research since phenomena and reactions are observed which cannot be investigated because the overall comparison of effects is the main objective and must be pursued in order to obtain conclusions. Very often the search for the explanation of an observation not related to the main problem is of greater interest and greater value than the original objective itself. By understanding the nature of an effect and its causes, relationships can be established between areas of research which may result in the development of theories which are applicable on a broad scale rather than to an individual aspect.

There were three major phenomena observed during the conduct of these investigations which, in the opinion of the author, warrant further study.

The first of these phenomena pertains to the extension of the adjustment phase of bacterial growth on chicken meat, which resulted from the exposure of the chicken meat to ionizing radiations. Further studies of this phenomenon directed toward determining the cause could result in the discovery of the compound or compounds responsible for the extended lag. Such research offers a remote possibility that a new microbial inhibitor might be discovered. Moreover, such an investigation might add information to the present knowledge of compounds formed in food by irradiation treatment.

There is a school of thought in radiation research which might cause one to suspect that long-lived organic peroxide radicals, which may be produced in foods such as chicken meat by radiation treatment, may be responsible for the extension of the adjustment phase of microorganisms grown upon such food. It is recognized that there are numerous other substances which may be suspected as well.

An approach to the problem may be made through the development of a simplified system to replace the complexities presented by the chicken muscle, followed by a study of the effect of the addition of individual substances to the simplified system.

The second phenomenon considered worthy of further study is also related to the extension of the adjustment phase of microorganisms when grown on chicken meat previously exposed to ionizing radiations. This problem can best be presented in the form of a question. Why should a bacterial inhibitor which decays as time elapses not demonstrate a radiation dose rate dependency during the period in which it is being formed? The answer to this question may add to what is already known concerning the mode of action of ionizing energy.

The observations made during these investigations regarding the formation of inhibitory substances indicate that the equilibrium of the system may be shifted in one direction while the ionizing energy is being applied. As long as the equilibrium is in this "build-up" direction there seems to be no decay of the unstable inhibitory substance or substances which are formed.

After the energy source is removed the unstable inhibitory substance appears to diminish in effectivity and becomes increasingly less significant as time elapses.

Research should be performed to determine whether the observed inhibitor "build-up" effect is attributable to the hypothesized equilibrium shift caused by the energy input. If the inhibitor "build-up" is found to be an equilibrium shift phenomenon, further studies should be carried out to determine the threshold dose rate, if one exists, which will result in sufficient production of the inhibitory substance to prevent microbial growth. Studies should also be performed to determine the effect of this same system upon enzymes. Should the threshold dose rate level be relatively low and should the inhibition be effective on enzyme systems as well as bacteria, there exists a possibility that the system could be utilized in the extension of the storage life of foods and other perishable commodities. Such a system would open many areas for the utilization of low dose rate isotopic sources of ionizing radiations.

The third phenomenon which appears to require further investigation is the growth rate differences which were observed when Staph. aureus was cultured on chicken meat which had been previously treated with heat or ionizing radiations. In the study presented in this thesis it was evident that heat processed, and especially irradiated chicken meat is a less suitable substrate for the support of growth of Staph. aureus. It is therefore possible that, where a culture of Staph. aureus was once able to flourish it could now reproduce only very slowly due to

processing of the chicken meat.

If research were carried out to determine the existence and identification of bacterial growth rate controlling substances, then it may become possible by chemical analysis of a food product to predict the organisms most likely to flourish on any particular food product. It may then be possible to develop specific methods for control of these organisms in each product.

APPENDICES

APPENDIX I

TABULATED DATA

A. Data accumulated during studies to determine the adequacy of a simulated chicken medium

TABLE 17

Growth response of Escherichia coli on various gelling agents at 37°C

Time (hours)	Log_{10} Number of Organisms Per Disc*					
	Bacto Agar		Seakem SK 204		Purified Agar	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
0	--	--	--	--	4.2967	--
1	--	--	--	--	4.0969	4.2788
2	--	--	--	--	3.7853	4.0086
3	--	--	--	--	3.5185	3.7853
4	5.2878	5.1239	6.1461	6.0969	3.3424	3.6721
4 1/2	--	--	--	--	--	--
5	--	--	--	--	3.0792	3.3979
5 1/2	--	--	--	--	--	--
6	6.3096	6.1732	7.4265	7.4265	2.9542	3.2430
6 1/2	--	--	--	--	--	--
7	--	--	--	--	--	--
7 1/2	--	--	--	--	--	--
8	6.6990	6.7559	8.3617	8.4314	--	--
8 1/2	--	--	--	--	--	--
9	--	--	--	--	--	--
24	7.7853	--	9.1761	9.1847	--	--
26	7.7993	--	9.1553	9.1847	--	--
28	7.7404	--	9.2041	9.2041	--	--

*Each value in the table is the log of the mean of two discs. The mean of each disc was previously calculated from three individual plate counts in TGE agar.

TABLE 18

Growth response of *Escherichia coli* on various media at 37°C

Raw Chicken

Incubation time (hrs.)	TGE agar plate count	Mean of three plate counts (per disc)	Mean of three sets of plate counts (per disc)	Log_{10} Mean
0	172, 173, 161	1.69×10^4		
0	175, 173, 158	1.69×10^4	1.64×10^4	4.2148
0	148, 165, 149	1.54×10^4		
1 1/2	101, 100, 112	1.04×10^4		
1 1/2	191, 171, 196	1.86×10^4	1.54×10^4	4.1875
1 1/2	172, 191, 157	1.73×10^4		
3	224, 242, 232	2.33×10^4		
3	57, 56, 45	5.3×10^4	3.20×10^4	4.5051
3	197, 205, 189	1.97×10^5		
4	112, 90, 99	1.00×10^4		
4	28, 34, 29	3.0×10^4	6.0×10^4	4.7782
4	59, 52, 42	5.1×10^4		
5	98, 108, 73	9.3×10^5		
5	265, 262, 263	2.63×10^4	1.49×10^5	5.1732
5	79, 105, 86	9.0×10^5		
6	31, 49, 33	3.8×10^6		
6	152, 156, 163	1.57×10^5	9.2×10^5	5.9638
6	68, 81, 73	7.4×10^6		
7	240, 226, 237	2.34×10^6		
7	164, 182, 169	1.72×10^6	1.63×10^6	6.2122
7	81, 72, 93	8.2×10^6		
8	87, 82, 86	8.5×10^7		
8	118, 93, 91	1.01×10^6	8.0×10^6	6.9031
8	56, 54, 51	5.3×10^7		
9	127, 127, 142	1.32×10^6		
9	43, 46, 42	4.4×10^7	1.63×10^7	7.2122
9	304, 282, 249	3.12×10^7		
10	18, 5, 9	1.1×10^7		
10	76, 69, 64	7.0×10^7	3.9×10^7	7.5911
10	42, 30, 36	3.6×10^7		

TABLE 19

Raw chicken - heated to 45°C followed by chilling prior to inoculation

Incubation time (hours)	TGE agar plate count	Mean of three plate counts (per disc)	Mean of two sets of plate counts (per disc)	Log_{10} mean
0	153, 129, 151	1.44×10^4	1.50×10^4	4.1761
0	172, 163, 132	1.56×10^4		
1 1/2	75, 83, 87	8.2×10^3	1.25×10^4	4.0969
1 1/2	166, 169, 168	1.68×10^4		
3	40, 38, 42	4.0×10^4	3.75×10^4	5.5740
3	40, 34, 30	3.5×10^4		
4	38, 31, 34	3.4×10^5	3.4×10^5	5.5315
4	311, 379, 331	3.40×10^5		
5	164, 135, 157	1.52×10^6	1.44×10^6	6.1584
5	92, 118, 95	1.35×10^6		
6	284, 279, 297	2.87×10^6	2.62×10^6	6.4183
6	216, 216, 231	2.36×10^6		
7	145, 152, 166	1.54×10^7	1.56×10^7	7.1931
7	164, 168, 142	1.58×10^7		
8	325, 341, 374	3.26×10^7	3.58×10^7	7.5539
8	35, 42, 40	3.9×10^7		
9	43, 36, 55	4.5×10^7	4.35×10^7	7.6385
9	47, 30, 49	4.2×10^7		
10	161, 163, 176	1.67×10^8	1.46×10^8	8.1644
10	116, 130, 125	1.24×10^8		

TABLE 20

Simulated raw chicken medium

Incubation time (hours)	SET 1 TGE agar plate counts	SET 1 Mean of three plate counts (per disc)	SET 2 TGE agar plate counts	SET 2 Mean of three plate counts (per disc)	SUMMARY Mean of three plate counts (per disc)	SUMMARY Log_{10} mean
0	164, 185, 190	1.79×10^4	155, 166, 137	1.53×10^4	1.68×10^4	4.2253
0	156, 150, 160	1.55×10^4	180, 175, 195	1.83×10^4		
1 1/2	130, 135, 137	1.34×10^4	139, 115, 110	1.21×10^4	1.54×10^4	4.1875
1 1/2	243, 309, 226	2.59×10^5	106, 110, 91	1.02×10^5		
3	145, 134, 145	1.41×10^5	236, 227, 265	2.43×10^5	1.65×10^5	5.2175
3	133, 152, 128	1.38×10^5	121, 151, 146	1.39×10^5		
4	90, 103, 83	9.2×10^5	103, 114, 121	1.13×10^6	1.07×10^6	6.0294
4	111, 101, 116	1.09×10^6	117, 117, 105	1.13×10^6		
5	48, 47, 57	5.1×10^6	604, 538, 656	5.99×10^6	5.36×10^6	6.7292
5	48, 60, 52	5.3×10^7	570, 470, 475	5.05×10^7		
6	107, 125, 118	1.17×10^7	157, 171, 160	1.63×10^7	1.53×10^7	7.1847
6	129, 137, 120	1.29×10^7	221, 209, 212	2.14×10^7		
7	264, 275, 294	2.78×10^7	850, 685, 855	7.95×10^7	5.01×10^7	7.6998
7	43, 47, 60	5.0×10^7	445, 405, 435	4.30×10^8		
8	95, 93, 101	9.6×10^7	265, 205, 212	2.27×10^8	1.61×10^8	8.2068
8	127, 139, 123	1.29×10^8	216, 171, 185	1.91×10^8		
9	31, 44, 38	3.8×10^8	39, 28, 31	3.3×10^8	4.2×10^8	8.6232
9	34, 45, 52	4.4×10^8	52, 56, 47	5.2×10^8		

B. Data accumulated during the adjustment phase of growth of Escherichia coli on various media at 37°C

TABLE 21

Raw chicken meat

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts (per disc)	SET 1 N/N_o mean x 100	SET 2 TGE agar plate count	SET 2 Mean of three plate counts (per disc)	SET 2 N/N_o mean x 100
0	144, 135, 176	1.52×10^4	102.4	150, 138, 141	1.43×10^4	95.3
0	143, 157, 134	1.45×10^4	97.6	168, 142, 160	1.57×10^4	104.7
0						
1/2	92, 69, 78	8.0×10^3	53.9	93, 78, 76	8.2×10^3	54.7
1/2	75, 58, 48	6.0×10^3	40.4	63, 52, 60	5.8×10^3	38.7
1/2						
1	174, 161, 167	1.67×10^4	112.5	104, 116, 106	1.09×10^4	72.7
1	116, 118, 148	1.27×10^4	85.5	119, 89, 81	9.6×10^3	64.0
1						
1 1/2	94, 85, 86	8.8×10^3	59.3	87, 96, 79	8.7×10^3	58.0
1 1/2	152, 146, 157	1.52×10^4	102.4	14, 24, 16	1.8×10^3	12.0
1 1/2						
2	225, 247, 230	2.34×10^4	157.6	248, 224, 196	2.23×10^4	148.7
2	163, 122, 130	1.38×10^4	92.9	172, 177, 179	1.76×10^4	117.3
2						
2 1/2	162, 147, 170	1.60×10^4	107.7	332, 344, 324	3.33×10^4	222.0
2 1/2	358, 328, 381	3.56×10^4	239.7	31, 30, 39	3.3×10^4	222.0
2 1/2						
3	65, 72, 68	6.8×10^4	457.9	49, 36, 42	4.2×10^4	280.0
3	99, 93, 99	9.7×10^4	653.2	54, 59, 55	5.6×10^4	373.3
3						

(continued)

TABLE 21

Raw chicken meat

Incubation time (hours)	SET 3 TGE agar plate count	SET 3 Mean of three plate counts (per disc)	SET 3 N/N_o mean x 100	SET 4 TGE agar plate count	SET 4 Mean of three plate counts (per disc)	SET 4 N/N_o mean x 100
0	241, 211, 257	2.36×10^4	105.8	contaminated		
0	226, 233, 238	2.32×10^4	104.0	208, 204, 193	2.02×10^4	100
0	209, 206, 190	2.02×10^4	90.6			
1/2	162, 154, 174	1.63×10^4	73.1	153, 172, 145	1.57×10^4	77.7
1/2	116, 119, 127	1.21×10^4	54.3	139, 172, 144	1.52×10^4	75.3
1/2	151, 168, 145	1.55×10^3	69.5			
1	57, 60, 42	5.3×10^4	23.8	132, 176, 179	1.62×10^4	80.2
1	122, 88, 101	1.04×10^3	46.6	154, 177, 147	1.59×10^4	78.7
1	45, 35, 47	4.2×10^4	18.8			
1 1/2	145, 132, 127	1.35×10^4	60.5	64, 65, 71	6.7×10^3	33.2
1 1/2	191, 165, 164	1.73×10^3	77.6	132, 134, 110	1.25×10^4	61.9
1 1/2	53, 39, 34	4.2×10^3	18.8			
2	77, 81, 82	8.0×10^4	35.9	contaminated		
2	184, 193, 245	2.07×10^4	92.8	189, 193, 190	1.91×10^4	94.6
2	111, 128, 153	1.31×10^4	58.7			
2 1/2	166, 156, 184	1.69×10^4	75.8	60, 48, 48	5.2×10^4	257.4
2 1/2	304, 277, 334	3.05×10^4	136.8	30, 28, 35	3.1×10^4	153.5
2 1/2	Lab. accident					
3	225, 213, 203	2.14×10^4	96.0			
3	87, 81, 96	8.8×10^4	394.6			
3	199, 183, 176	1.86×10^4	83.4			

(continued)

TABLE 21

Raw chicken meat

Incubation time (hours)	TGE agar plate count	SET 5 Mean of three plate counts (per disc)	N/N_o mean x 100	SUMMARY N/N_o x100 mean of five sets	Log_{10} N/N_o x 100
0	191, 196, 201	1.96×10^4	108.6		
0	185, 151, 158	1.65×10^4	91.4	100	2.0000
0					
1/2	96, 111, 97	1.01×10^4	56.0		
1/2	136, 131, 104	1.24×10^4	68.7	60.2	1.7796
1/2					
1	170, 147, 176	1.64×10^4	90.9		
1	164, 183, 203	1.83×10^4	101.4	70.5	1.8482
1					
1 1/2	270, 240, 234	2.48×10^4	137.4		
1 1/2	149, 124, 134	1.36×10^4	75.4	63.3	1.8014
1 1/2					
2	219, 192, 199	2.03×10^4	112.5		
2	308, 276, 213	2.66×10^4	147.4	105.8	2.0245
2					
2 1/2	308, 299, 312	3.06×10^4	169.5		
2 1/2	311, 304, 326	3.14×10^4	174.0	175.8	2.2450
2 1/2					
3	66, 61, 59	6.2×10^4	343.5		
3	134, 140, 122	1.32×10^5	731.3	379.2	2.5789
3					

TABLE 22

Heat processed chicken meat

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts per disc	SET 1 N/N_o mean x 100	SET 2 TGE agar plate count	SET 2 Mean of three plate counts per disc	SET 2 N/N_o mean x 100
0	102, 103, 102	1.02×10^5	93.6	42, 42, 52	4.6×10^4	100
0	110, 123, 114	1.16×10^5	106.4	contaminated		
1/2	115, 118, 116	1.16×10^5	106.4	258, 276, 270	2.68×10^4	58.3
1/2	108, 111, 131	1.17×10^5	107.3	contaminated		
1	127, 143, 133	1.34×10^5	122.9	27, 25, 22	2.5×10^4	54.4
1	132, 113, 129	1.25×10^5	114.7	42, 37, 35	3.8×10^4	82.6
1 1/2	80, 87, 94	8.7×10^4	79.8	47, 35, 49	4.3×10^4	93.5
1 1/2	77, 87, 89	8.4×10^4	77.1	41, 52, 35	4.3×10^4	93.5
2	93, 98, 79	9.0×10^4	82.6	74, 73, 86	7.8×10^4	169.6
2	179, 134, 156	1.56×10^5	143.1	contaminated		
2 1/2	145, 123, 140	1.02×10^5	93.6	91, 97, 88	8.5×10^4	184.8
2 1/2	166, 153, 158	1.19×10^5	109.2	171, 174, 173	1.73×10^5	376.1
3	218, 214, 203	2.12×10^5	194.5	169, 181, 172	1.74×10^5	378.3
3	44, 38, 47	4.3×10^5	394.5	272, 288, 271	2.77×10^5	602.2

(continued)

TABLE 22

Heat processed chicken meat

Incubation time (hours)	SET 3 TGE agar plate count	Mean of three plate counts per disc	N/N_o mean x 100	SUMMARY $N/N_o x100$ mean of three sets	Log_{10} $N/N_o x100$
0	247, 251, 250	$2.49x10^4$	101.2	100	2.0000
0	253, 245, 231	$2.43x10^4$	98.8		
1/2	180, 181, 181	$1.81x10^4$	73.6	84.1	1.9248
1/2	184, 178, 190	$1.84x10^4$	74.8		
1	214, 190, 196	$2.00x10^4$	81.3	91.5	1.9614
1	240, 222, 225	$2.29x10^4$	93.1		
1 1/2	198, 212, 202	$2.04x10^4$	82.9	84.3	1.9258
1 1/2	206, 197, 198	$1.94x10^4$	78.9		
2	40, 34, 31	$3.5 x10^4$	142.3	129.1	2.1109
2	244, 294, 261	$2.66x10^4$	108.1		
2 1/2	46, 53, 45	$4.8 x10^4$	195.1	193.0	2.2856
2 1/2	52, 54, 41	$4.9 x10^4$	199.2		
3	72, 72, 62	$6.9 x10^5$	280.5	407.9	2.6106
3	147, 152, 141	$1.47x10^5$	597.6		

TABLE 23

Irradiated chicken meat
Dose: $1x10^6$ rads of ionizing radiations

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts (per disc)	SET 1 $N/N_o x100$ mean	SET 2 TGE agar plate count	SET 2 Mean of three plate counts (per disc)	SET 2 $N/N_o x100$ mean
0	180, 205, 199	$1.95x10^4$	92.6	190, 170, 152	$1.71x10^4$	101.5
0	239, 230, 210	$2.26x10^4$	107.4	162, 177, 160	$1.66x10^4$	98.5
1/2	223, 193, 185	$2.00x10^4$	95.0	134, 130, 140	$1.35x10^4$	80.1
1/2	189, 195, 188	$1.91x10^4$	90.7	146, 146, 145	$1.46x10^3$	86.7
1	110, 111, 124	$1.15x10^4$	54.6	77, 98, 66	$8.0 x10^3$	47.5
1	165, 159, 154	$1.59x10^4$	75.5	80, 77, 74	$7.7 x10^3$	45.7
1 1/2	138, 200, 145	$1.61x10^4$	76.5	105, 107, 95	$1.02x10^4$	60.5
1 1/2	163, 162, 197	$1.74x10^4$	82.7	125, * 134	$1.30x10^4$	77.2
2	156, 158, 142	$1.52x10^4$	72.2	174, 198, 151	$1.74x10^4$	103.3
2	170, 194, 179	$1.81x10^4$	86.0	142, 133, 129	$1.35x10^4$	80.1
2 1/2	216, 197, 182	$1.98x10^4$	94.1	242, 248, 231	$2.40x10^4$	142.4
2 1/2	132, 164, 142	$1.46x10^4$	69.4	161, 186, 178	$1.75x10^4$	103.9
3	305, 283, 262	$2.83x10^4$	134.4	35, 38, 38	$3.7 x10^4$	219.6
3	30, 36, 30	$3.2 x10^4$	152.0	33, 34, 24	$3.0 x10^4$	178.0

*contaminated

(continued)

TABLE 23

Irradiated chicken meat
Dose: $1x10^6$ rads of ionizing radiations

SUMMARY

Incubation time (hours)	$N/N_o x100$ Mean of three sets	Log_{10} $N/N_o x100$
0 0	100	2.0000
1/2 1/2	88.1	1.9450
1 1	55.8	1.7466
1 1/2 1 1/2	74.2	1.8704
2 2	85.4	1.9315
2 1/2 2 1/2	102.5	2.0107
3 3	171.0	2.2330

Irradiated chicken meat
Dose: 6×10^6 rads of ionizing radiations

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts (per disc)	SET 1 N/N_o mean x 100	SET 2 TGE agar plate count	SET 2 Mean of three plate counts (per disc)	SET 2 N/N_o mean x 100
0	148, 102, 143	1.31×10^4	112.0	166, 154, 163	1.61×10^4	106.6
0	117, 96, 95	1.03×10^4	88.0	145, 137, 140	1.41×10^4	93.4
1/2	104, 87, 116	1.02×10^4	87.2	115, 120, 137	1.24×10^4	82.1
1/2	114, 92, 112	1.06×10^4	90.6	129, 145, 138	1.37×10^4	90.7
1	93, 116, 98	1.02×10^4	87.2	100, 123, 90	1.04×10^4	68.9
1	113, 130, 117	1.20×10^3	102.6	173, 167, 151	1.67×10^3	110.6
1 1/2	88, 98, 96	9.4×10^3	80.3	60, 61, 56	5.9×10^4	39.1
1 1/2	75, 76, 92	8.1×10^3	69.2	106, 113, 105	1.08×10^4	71.5
2	98, 102, 84	9.5×10^3	81.2	104, 108, 110	1.07×10^3	70.9
2	42, 50, 68	5.3×10^3	45.3	65, 73, 57	6.5×10^3	43.1
2 1/2	49, 64, 61	5.8×10^3	49.6	46, 38, 38	4.1×10^3	27.2
2 1/2	83, 90, 84	8.6×10^3	73.5	76, 70, 86	7.7×10^3	51.0
3	64, 72, 66	6.7×10^3	57.3	78, 63, 74	7.2×10^3	47.7
3	79, 71, 77	7.6×10^3	65.0	31, 49, 48	4.3×10^3	28.5
3 1/2	70, 59, 55	3.1×10^3	26.5	80, 72, 53	6.8×10^3	45.0
3 1/2	42, 34, 39	1.9×10^3	16.2	43, 33, 34	3.7×10^3	24.5
4	57, 54, 42	5.1×10^3	43.6	41, 40, 45	4.2×10^3	27.8
4	15, 11, 17	1.4×10^3	12.0	44, 36, 43	4.1×10^3	27.1
4 1/2	23, 27, 24	2.5×10^3	21.4	65, 57, 47	5.6×10^3	37.1
4 1/2	18, 20, 19	1.9×10^3	16.2	71, 51, 68	6.3×10^3	41.7
5	21, 21, 20	2.1×10^3	18.0	85, 73, 92	8.3×10^4	55.0
5	36, 20, 27	2.8×10^3	23.9	211, 227, 190	2.09×10^4	138.4
6	35, 41, 30	3.5×10^3	29.9	55, 59, 55	5.6×10^4	332.4
6	67, 60, 51	5.9×10^3	50.4	128, 133, 129	1.30×10^5	77.2
7	70, 61, 67	6.9×10^3	59.0	194, 208, 217	2.06×10^4	1225.5
7	86, 74, 74	7.8×10^3	66.7	229, 226, 214	2.23×10^4	1323.4

(continued)

TABLE 24

Irradiated chicken meat
Dose: 6×10^6 rads of ionizing radiations

Incubation time (hours)	TGE agar plate count	SET 3 Mean of three plate counts (per disc)	N/N_o mean x 100	SUMMARY $N/N_o \times 100$ Mean of three sets	Log_{10} $N/N_o \times 100$
0	121, 102, 115	1.13×10^4	95.8	100	2.0000
0	129, 118, 122	1.23×10^4	104.2		
1/2	107, 110, 117	1.11×10^4	94.1	92.1	1.9643
1/2	130, 115, 135	1.27×10^4	107.6		
1	150, 163, 128	1.47×10^3	124.6	95.0	1.9777
1	102, 78, 90	9.0×10^3	76.3		
1 1/2	109, 88, 100	9.9×10^3	83.9	69.5	1.8420
1 1/2	89, 82, 86	8.6×10^3	72.9		
2	98, 70, 73	8.0×10^3	67.8	66.9	1.8254
2	109, 98, 123	1.10×10^4	93.2		
2 1/2	71, 53, 56	6.0×10^3	50.9	51.9	1.7152
2 1/2	76, 73, 61	7.0×10^3	59.3		
3	44, 44, 52	4.7×10^3	39.8	48.2	1.6830
3	62, 60, 59	6.0×10^3	50.9		
3 1/2	22, 38, 46	3.5×10^3	29.7	30.9	1.4900
3 1/2	64, 40, 49	5.1×10^3	43.2		
4	22, 38, 30	3.0×10^3	25.4	26.6	1.4249
4	24, 28, 33	2.8×10^3	23.7		
4 1/2				29.1	1.4624
4 1/2					
5	36, 34, 41	3.7×10^3	31.4	47.0	1.6721
5	19, 19, 17	1.8×10^3	15.3		
6	66, 87, 77	7.7×10^3	65.3	105.5	2.0233
6	88, 84, 103	9.2×10^4	78.0		
7	66, 56, 57	6.0×10^3	508.5	540.7	2.7330
7	75, 63, 82	7.3×10^3	62.1		

C. Data accumulated during the logarithmic phase of growth of Escherichia coli on various media at 37°C

TABLE 25

Tryptone glucose extract broth

Set number	Incubation time (hours)	TGE agar plate count	No. of organisms (per ml.)	Log_{10} number of organisms per ml.
1	4 1/2	42	4.2×10^4	4.6232
	4 1/2	50	5.0×10^4	4.6990
	5	107	1.07×10^5	5.0294
	5	125	1.25×10^5	5.0969
	6	501	5.01×10^5	5.6998
	6	431	4.31×10^5	5.6345
	6 1/2	175	1.75×10^6	6.2430
	6 1/2	100	1.00×10^6	6.0000
	7	377	3.77×10^6	6.5763
	7	433	4.33×10^6	6.6365
	7 1/2	126	1.26×10^7	7.1004
	7 1/2	111	1.11×10^7	7.0453
	8	266	2.66×10^7	7.4166
	8	261	2.61×10^7	7.4249
	8 1/2	53	5.3×10^7	7.7243
	8 1/2	42	4.2×10^7	7.6232
	9	82	8.2×10^7	7.9138
	9	73	7.3×10^7	7.8633
2	5	36	3.6×10^5	5.5563
	5	48	4.8×10^5	5.6812
	5	54	5.4×10^5	5.7324
	5	70	7.0×10^5	5.8451
	5	54	5.4×10^5	5.7324
	6	17	1.7×10^6	6.2304
	6	42	4.2×10^6	6.6232
	6	33	3.3×10^6	6.5185
	6	42	4.2×10^6	6.6232
	6	28	2.8×10^7	6.4472
	7	263	2.63×10^7	7.4200
	7	266	2.66×10^7	7.4249
	7	285	2.85×10^7	7.4548
	7	246	2.46×10^7	7.3909
	7	268	2.68×10^7	7.4281
	8	87	8.7×10^8	7.9395
	8	129	1.29×10^8	8.1106
	8	110	1.10×10^8	8.0414
	8	103	1.03×10^8	8.0128
	8	100	1.00×10^8	8.0000

TABLE 26

Tryptone glucose extract agar

Set number	Incubation time (hours)	TGE agar plate count	No. of organisms (per disc)	Log_{10} number of organisms per disc
1	4	41	4.3×10^5	5.6128
	4	44	4.4×10^5	5.6435
	4 1/2	135	1.35×10^6	6.1303
	4 1/2	157	1.57×10^6	6.1959
	5	205	2.05×10^6	6.3118
	5	208	2.08×10^6	6.3181
	5 1/2	38	3.8×10^6	6.5798
	5 1/2	51	5.1×10^7	6.7076
	6	139	1.39×10^7	7.1430
	6	120	1.20×10^7	7.0792
	6 1/2	331	3.31×10^7	7.5198
	6 1/2	396	3.96×10^7	7.5977
	7	65	6.5×10^7	7.8129
	7	71	7.1×10^8	7.8513
	7 1/2	339	3.39×10^8	8.5302
	7 1/2	332	3.32×10	8.5211
2	5 1/2	235	2.35×10^6	6.3711
	5 1/2	162	1.62×10^6	6.2095
	6	73	7.3×10^6	6.8633
	6	69	6.9×10^7	6.8388
	6 1/2	109	1.09×10^7	7.0374
	6 1/2	113	1.13×10^7	7.0531
	7	179	1.79×10^7	7.2529
	7	251	2.51×10^7	7.3997
	7 1/2	30	3.0×10^7	7.4771
	7 1/2	43	4.3×10^7	7.6721
	8	76	7.6×10^7	7.8808
	8	78	7.8×10^8	7.8921
	8 1/2	169	1.69×10^8	8.2279
	8 1/2	188	1.88×10^9	8.2742
	9	142	1.42×10^9	9.1523
	9	121	1.21×10^9	9.0828
3	4	121	1.21×10^5	5.0828
	4	113	1.13×10^5	5.0531
	4 1/4	218	2.18×10^5	5.3385
	4 1/4	178	1.78×10^5	5.2504
	4 1/2	33	3.3×10^5	5.5185
	4 1/2	40	4.0×10^5	5.6021
	4 3/4	39	3.9×10^5	5.5911
	4 3/4	49	4.9×10^5	5.6902

(continued)

TABLE 26

Tryptone glucose extract agar

Set Number	Incubation time (hours)	TGE agar plate count	No. of organisms (per disc)	Log_{10} number of organisms per disc
	5	126	1.26×10^6	6.1004
	5	98	9.8×10^5	5.9912
	5 1/2	30	3.0×10^6	6.4771
	5 1/2	27	2.7×10^6	6.4314
	5 3/4	32	3.2×10^6	6.5051
	5 3/4	58	5.8×10^6	6.7634
	6	69	6.9×10^6	6.8388
	6	76	7.6×10^6	6.8808
	6 1/4	136	1.36×10^7	7.1335
	6 1/4	129	1.29×10^7	7.1106
	6 1/2	175	1.75×10^7	7.2430
	6 1/2	193	1.93×10^7	7.2856
	7	280	2.80×10^7	7.4472
	7	258	2.58×10^7	7.4116
	7 1/2	42	4.2×10^7	7.6232
	7 1/2	39	3.9×10^8	7.5911
	8	140	1.40×10^8	8.1461
	8	141	1.41×10^8	8.1492

TABLE 27

Raw chicken meat

Set number	Incubation time (hours)	TGE agar plate count	Mean of three plate counts (per disc)	Log_{10} mean
1	4	164, 178, 139	1.60×10^5	5.2041
	4	250, 274, 273	2.66×10^5	5.4249
	4	171, 167, 192	1.77×10^5	5.2480
	5	209, 218, 226	2.27×10^5	5.3560
	5	192, 213, 190	1.98×10^5	5.2967
	5	32, 32, 32	3.2×10^6	5.5051
	6	159, 190, 180	1.76×10^6	6.2455
	6	160, 163, 153	1.59×10^6	6.2014
	6	215, 246, 222	2.28×10^6	6.3579
	7	56, 58, 53	5.6×10^6	6.7482
	7	42, 49, 34	4.2×10^6	6.6232
	7	63, 70, 70	6.8×10^7	6.8325
	8	133, 133, 102	1.23×10^7	7.0899
	8	42, 31, 31	3.5×10^7	7.5441
	8	232, 226, 224	2.27×10^7	7.3560
2	4	112, 90, 99	1.00×10^5	5.0000
	4	28, 34, 29	3.0×10^4	4.4771
	4	59, 52, 42	5.1×10^4	4.7076
	5	98, 108, 73	9.3×10^5	4.9685
	5	152, 156, 163	2.63×10^5	5.4200
	5	68, 81, 73	9.0×10^5	4.9542
	6	31, 49, 33	3.8×10^6	5.5798
	6	152, 156, 163	1.57×10^6	6.1959
	6	68, 81, 73	7.4×10^6	5.8692
	7	240, 226, 237	2.34×10^6	6.3692
	7	164, 182, 169	1.72×10^6	6.2355
	7	81, 72, 93	8.2×10^6	5.9138
	8	87, 82, 86	8.5×10^7	6.9294
	8	118, 93, 91	1.01×10^7	7.0043
	8	56, 54, 51	5.3×10^6	6.7243

TABLE 28

Heat processed chicken meat

Set number	Incubation time (hours)	TGE agar plate count	Mean of three plate counts (per disc)	Log_{10} mean
1	3	218, 214, 203	2.12×10^5	5.3263
	3	44, 38, 47	4.3×10^5	5.6335
	4	156, 146, 131	1.44×10^6	6.1584
	4	193, 169, 173	1.78×10^6	6.2504
	5	128, 158, 149	1.45×10^7	7.1614
	5	112, 105, 104	1.07×10^7	7.0294
	6	35, 46, 30	3.7×10^7	7.5682
	6	55, 63, 57	5.8×10^8	7.7634
	7	124, 153, 116	1.31×10^8	8.1173
	7	129, 97, 94	1.07×10^8	8.0294
	8	86, 76, 68	7.7×10^8	8.8865
	8	53, 75, 72	6.7×10^8	8.8261
2	3	72, 72, 62	6.9×10^4	4.8383
	3	147, 152, 141	1.47×10^5	5.1673
	4	94, 107, 111	1.04×10^6	6.0170
	4	88, 97, 102	9.6×10^5	5.9823
	5	41, 28, 43	3.7×10^6	6.5682
	5	51, 57, 43	5.0×10^6	6.6990
	7	126, 122, 107	1.18×10^8	8.0719
	7	167, 185, 170	1.74×10^8	8.2405
	8	47, 55, 49	5.0×10^8	8.6990
	8	37, 33, 27	3.2×10^8	8.5051

TABLE 29

Irradiated chicken meat
Dose: 1×10^6 rads of ionizing radiations*

Set number	Incubation time (hours)	TGE agar plate count	Mean of three plate counts (per disc)	Log_{10} mean
1	3	35, 38, 38	3.7×10^4	4.5682
	3	33, 32, 24	3.0×10^4	4.4771
	4	205, 192, 192	1.96×10^5	5.2923
	4	250, 277, 286	2.71×10^5	5.4330
	5	56, 48, 47	5.0×10^5	5.6990
	5	80, 88, 94	8.7×10^5	5.9395
	6	60, 37, 50	4.9×10^6	6.6902
	6	34, 42, 40	3.9×10^6	6.5911
	7	103, 104, 98	1.02×10^7	7.0086
	7	202, 174, 170	1.82×10^7	7.2601
2	3	305, 283, 262	2.83×10^4	4.4518
	3	30, 36, 30	3.2×10^4	4.5051
	4	59, 74, 62	6.5×10^4	4.8129
	4	88, 88, 69	8.2×10^4	4.9138
	5	80, 97, 76	8.4×10^4	5.9243
	5	112, 99, 103	1.05×10^6	6.0212
	6	69, 68, 70	6.9×10^6	6.8388
	6	244, 250, 287	2.60×10^7	6.4150
	7	153, 159, 163	1.58×10^7	7.1987
	7	125, 134, 141	1.33×10^7	7.1239
	8	62, 65, 65	6.4×10^7	7.8062
	8	26, 30, 42	3.3×10^7	7.5185

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 30

Irradiated Chicken Meat
Dose: 6×10^6 rads of Ionizing Radiations*

Set number	Incubation time (hours)	TGE agar plate count	Mean of three plate counts (per disc)	Log_{10} mean
1	5	36, 34, 41	3.7×10^3	3.5682
	5	19, 19, 17	1.8×10^3	3.2553
	6	66, 87, 77	7.7×10^3	3.8865
	6	88, 84, 103	9.2×10^4	3.9638
	7	66, 56, 57	6.0×10^3	4.7782
	7	75, 63, 82	7.3×10^5	3.8633
	8	303, 287, 268	2.86×10^5	5.4564
	8	100, 106, 111	1.06×10^6	5.0253
	9	212, 225, 215	2.17×10^5	6.3365
	9	63, 78, 89	7.7×10^7	5.8865
	11	123, 147, 113	1.28×10^7	7.1072
	11	77, 98, 81	8.5×10^6	6.9294
2	5	85, 73, 92	8.3×10^4	3.9191
	5	211, 227, 190	2.09×10^4	4.3201
	6	55, 59, 55	5.6×10^4	4.7482
	6	128, 133, 129	1.30×10^5	4.1139
	7	194, 208, 217	2.06×10^4	5.3139
	7	229, 226, 214	2.23×10^5	4.3483
	8	60, 50, 53	5.4×10^5	5.7324
	8	47, 42, 48	4.6×10^6	5.6628
	9	35, 47, 26	3.6×10^6	6.5563
	9	310, 298, 304	3.04×10^6	6.4829
	10	99, 72, 58	7.6×10^6	6.8808
	10	82, 78, 76	7.9×10^7	6.8976
	11	31, 38, 28	3.2×10^7	7.5051
	11	42, 44, 47	4.4×10^7	7.6435

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

D. Data accumulated during the stationary phase of growth of Escherichia coli on various media at 37°C

TABLE 31

Tryptone glucose extract agar

Incubation time (hours)	TGE agar plate count	Mean of three plate counts (org/disc)	Overall mean of plate counts (org/disc)	Log_{10} Mean
24	206, 225, 213	2.15×10^{10}	2.22×10^{10}	10.3464
24	219, 232, 232	2.28×10^{10}		
26	233, 185, 216	2.11×10^{10}	2.03×10^{10}	10.3075
26	195, 210, 181	1.95×10^{10}		
28	198, 181, 181	1.87×10^{10}	1.92×10^{10}	10.2833
28	204, 194, 190	1.96×10^{10}		

TABLE 32

Raw chicken meat

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts org/disc	SET 2 TGE agar plate count	SET 2 Mean of three plate counts org/disc	SET 3 TGE agar plate count	SET 3 Mean of three plate counts org/disc
22					82, 64, 68	7.1×10^9
22					50, 54, 46	5.0×10^9
24			80, 83, 81	8.1×10^9	98, 108, 101	1.02×10^{10}
24			71, 94, 90	8.5×10^{10}	88, 116, 96	1.00×10^9
26			159, 166, 165	1.63×10^{10}	101, 77, 89	9.2×10^{10}
26			105, 108, 108	1.07×10^{10}	129, 123, 157	1.36×10^{10}
28	33, 37, 32	3.4×10^9	125, 122, 126	1.23×10^{10}	106, 121, 119	1.15×10^{10}
28	38, 35, 29	3.4×10^9	165, 174, 185	1.75×10^{10}	167, 188, 191	1.82×10
30	184, 188, 164	1.79×10^9	157, 151, 174	1.61×10^{10}		
30	71, 92, 81	8.1×10^9	97, 100, 83	9.3×10^{10}		
32	75, 79, 93	8.2×10^9	134, 152, 168	1.51×10^{10}		
32	106, 91, 112	1.03×10^9	212, 180, 193	1.95×10		
34	97, 60, 77	7.8×10^{10}				
34	111, 113, 94	1.06×10^{10}				

(continued)

TABLE 32

Raw chicken meat

	SET 4		SUMMARY	
Incubation time (hours)	TGE agar plate count	Mean of three plate counts org/disc	Overall mean of plate counts org/disc	Log_{10} mean
22	98, 90, 79	8.9×10^9		
22	71, 70, 89	7.7×10^9	7.2×10^9	9.8573
24	89, 83, 92	8.8×10^9		
24	193, 177, *	1.85×10^{10}	1.07×10^{10}	10.0294
26	130, 136, 149	1.38×10^{10}		
26	176, 154, 176	1.69×10^{10}	1.34×10^{10}	10.1271
28	195, 229, 191	2.05×10^{10}		
28	38, 37, 41	3.7×10^{10}	2.31×10^{10}	10.3636
30				
30			1.28×10^{10}	10.1072
32				
32			1.10×10^{10}	10.0414
34				
34			9.2×10^9	9.9638

*Laboratory accident

TABLE 33

Heat processed chicken meat

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts org/disc	SET 2 TGE agar plate count	SET 2 Mean of three plate counts org/disc
20	29, 31, 28	2.9×10^{9}	35, 25, 26	2.9×10^{10}
20	141, 170, 158	1.56×10^{10}	30, 41, 30	3.4×10^{10}
22	312, 301, 321	3.11×10^{10}	95, 107, 100	1.01×10^{10}
22	453, 411, 399	4.21×10^{10}	111, 112, 101	1.08×10^{10}
24	369, 373, 392	3.78×10^{10}	42, 38, 48	4.3×10^{10}
24	258, 250, 260	2.56×10^{10}	33, 36, 38	3.6×10^{10}
26	260, 280, 270	2.70×10^{10}	35, 36, 32	3.4×10^{10}
26	240, 247, 242	2.43×10^{10}	42, 40, 48	4.3×10^{10}
28	392, 385, [illegible]	3.89×10^{10}	147, 136, 135	1.39×10^{10}
28			40, 47, 61	4.9×10^{10}
30				
30				
32				
32				

(continued)

TABLE 33

Heat processed chicken meat

Incubation time (hours)	SET 3 TGE agar plate count	SET 3 Mean of three plate counts org/disc	SUMMARY Overall mean of plate counts org/disc	SUMMARY Log_{10} mean
20			2.62×10^{10}	10.4183
20				
22			2.35×10^{10}	10.3711
22				
24	228, 222, 259	2.36×10^{10}	3.09×10^{10}	10.4900
24	194, 197, 198	1.96×10^{10}		
26	286, 287, 292	2.88×10^{10}	3.08×10^{10}	10.4886
26	280, 276, 275	2.77×10^{10}		
28	245, 219, 255	2.40×10^{10}	3.10×10^{10}	10.4914
28	288, 291, 297	2.92×10^{10}		
30	212, 270, 242	2.41×10^{10}	2.82×10^{10}	10.4502
30	309, 334, 326	3.23×10^{10}		
32	346, 368, 321	3.45×10^{10}	3.12×10^{10}	10.4942
32	284, 271, 248	2.78×10^{10}		

TABLE 34

Irradiated chicken
Dose: 1×10^6 rads of ionizing radiations

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts org/disc	SET 2 TGE agar plate count	SET 2 Mean of three plate counts org/disc	SUMMARY Overall mean of plate counts org/disc	SUMMARY Log_{10} mean
24			133, 162, 167	1.54×10^{10}		
24	136, 141, 120	1.32×10^{10}	168, 171, 169	1.69×10^{10}	1.52×10^{10}	10.1818
25	207, 225, 203	2.12×10^{10}	217, 214, 244	2.25×10^{10}		
25	178, 227, 188	1.98×10^{10}			2.12×10^{10}	10.3263
26	201, 167, 177	1.82×10^{10}				
26	270, 261, 250	2.60×10^{10}	192, 210, 188	1.97×10^{10}	2.13×10^{10}	10.3284
27	189, 183, 172	1.81×10^{10}	128, 110, 107	1.15×10^{10}		
27	219, 212, 211	2.14×10^{10}			1.70×10^{10}	10.2304
28	35, 49, 44	4.3×10^{10}	254, 216, 234	2.35×10^{10}		
28	219, 218, 206	2.14×10^{10}	303, 260, 280	2.81×10^{10}	2.43×10^{10}	10.3856
29			241, 278, 314	2.78×10^{10}	2.78×10^{10}	10.4440

TABLE 35

Irradiated chicken meat
Dose: 6×10^6 rads of ionizing radiations

Incubation time (hours)	SET 1 TGE agar plate count	SET 1 Mean of three plate counts org/disc	SET 2 TGE agar plate count	SET 2 Mean of three plate counts org/disc	SET 3 TGE agar plate count	SET 3 Mean of three plate counts org/disc
24	109, 103, 140	1.17×10^{10}	217, 248, 242	2.36×10^{10}	172, 163, 193	1.76×10^{10}
24	137, 85, 134	1.19×10^{10}	263, 259, 295	2.72×10^{10}		
26			234, 238, 222	2.31×10^{10}		
26			179, 198, 194	1.90×10^{10}		
27						
27						
28			244, 235, 253	2.44×10^{10}		
28			241, 272, 269	2.61×10^{10}		
30			317, 287, 331	3.12×10^{10}	327, 308, 272	3.02×10^{10}
30			194, 182, 176	1.84×10^{10}		
31	216, 190, 178	1.95×10^{10}				
31	143, 167, 161	1.57×10^{10}				
32	239, 202, 229	2.23×10^{10}			280, 302, 300	2.94×10^{10}
32	195, 181, 195	1.90×10^{10}				
33			306, 327, 349	3.27×10^{10}		
33			321, 296, 305	3.07×10^{10}		
34			301, 243, 279	2.74×10^{10}		
34			278, 278, 285	2.80×10^{10}		

(continued)

TABLE 35

Irradiated chicken meat
Dose: 6×10^6 rads of ionizing radiations

Incubation time (hours)	SET 4 TGE agar plate count	SET 4 Mean of three plate counts org/disc	SET 5 TGE agar plate count	SET 5 Mean of three plate counts org/disc	SET 6 TGE agar plate count	SET 6 Mean of three plate counts org/disc
24	199, 204, 219	2.07×10^{10}				
24					113, 105, 94	1.04×10^{10}
26	246, 246, 230	2.41×10^{10}	138, 147, 143	1.43×10^{10}	115, 125, 118	1.19×10^{10}
26	198, 206, 213	2.06×10^{10}			268, 228, 267	2.52×10^{10}
27					103, 94, 120	1.06×10^{10}
27						
28	206, 256, 223	2.28×10^{10}	131, 122, 116	1.23×10^{10}	235, 264, 293	2.64×10^{10}
28	229, 193, 209	2.10×10^{10}	208, 203, 198	2.03×10^{10}	262, 227, 272	2.54×10^{10}
30	241, 284, 257	2.61×10^{10}	252, 251, 251	2.51×10^{10}	271, 241, 249	2.54×10^{10}
30	289, 256, 257	2.67×10^{10}	231, 235, 223	2.30×10^{10}	249, 284, 253	2.62×10^{10}
31	282, 266, 278	2.75×10^{10}				
31	200, 201, 182	1.94×10^{10}				
32	322, 346, 286	3.18×10^{10}	234, 262, 214	2.37×10^{10}	277, 276, 244	2.66×10^{10}
32	243, 235, 242	2.40×10^{10}	227, 218, 208	2.18×10^{10}	266, 275, 258	2.66×10^{10}
33			273, 257, 273	2.68×10^{10}		
33			255, 250, 224	2.43×10^{10}		
34					333, 310, 339	3.27×10^{10}
34					308, 292, 292	2.97×10^{10}

(continued)

TABLE 35

Irradiated chicken meat
Dose: 6×10^6 rads of ionizing radiations

SET 7			SUMMARY	
Incubation time (hours)	TGE agar plate count	Mean of three plate counts org/disc	Overall mean of plate counts org/disc	Log_{10} mean
24				
24			1.88×10^{10}	10.2742
26				
26			2.02×10^{10}	10.3032
27	211, 195, 254	2.20×10^{10}		
27			2.20×10^{10}	10.3424
28				
28			2.12×10^{10}	10.3263
30	221, 303, 302	2.75×10^{10}		
30	275, 296, 313	2.95×10^{10}	2.58×10^{10}	10.4116
31	292, 286, 327	3.02×10^{10}		
31	251, 236, 255	2.47×10^{10}	2.05×10^{10}	10.3118
32	260, 273, 259	2.64×10^{10}		
32			2.47×10^{10}	10.3927
33				
33			2.86×10^{10}	10.4564
34				
34			2.95×10^{10}	10.4698

E. Data accumulated during the adjustment phase of growth of Staphylococcus aureus at 37°C in and on various media

TABLE 36

Brain heart infusion (at rest)

Incubation time (hours)	SET 1 BHI agar plate count	SET 1 Mean of three plate counts per ml.	SET 1 N/N_o mean x 100	SET 2 BHI agar plate count	SET 2 Mean of three plate counts per ml.	SET 2 N/N_o mean x 100
0	72, 83, 61	7.2×10^3	94.7	79, 110, 108	9.9×10^4	119.3
0	88, 74, 79	8.0×10^3	105.3	70, 77, 54	6.7×10^4	80.7
1/2	93, 90, 77	8.7×10^3	114.5	102, 92, 96	9.7×10^4	116.9
1/2	89, 80, 94	8.8×10^3	115.8	L. A.		
1	110, 103, 106	1.06×10^4	139.5	138, 120, 113	1.24×10^5	149.4
1	99, 96, 90	9.5×10^3	125.0	140, 123, 113	1.25×10^5	150.6
1 1/2	166, 146, 153	1.55×10^4	204.0	240, 228, 194	2.21×10^5	266.3
1 1/2	160, 167, 173	1.67×10^4	219.7	L.A. 235, 215	2.25×10^5	271.1
	SET 3			SET 4		
0	69, 72, 59	6.7×10^4	103.1	81, 73, 67	7.4×10^4	95.5
0	72, 64, 52	6.3×10^4	96.9	82, 66, 94	8.1×10^4	104.5
1/2	67, 52, 46	5.5×10^4	84.6	67, 87, 87	8.0×10^4	103.2
1/2	76, 64, 59	6.6×10^4	101.5	107, 117, 103	1.09×10^5	140.7
1	95, 77, 84	8.5×10^4	130.8	116, 93, 96	1.02×10^5	131.6
1	91, 94, 89	9.1×10^4	140.0	100, 106, 99	1.02×10^5	131.6
1 1/2	158, 110, 133	1.34×10^5	206.2	154, 166, 154	1.58×10^5	203.9
1 1/2	167, 195, 143	1.68×10^5	258.5	193, 169, 189	1.84×10^5	237.4

SUMMARY

	N/N_o x100 Mean of 4 sets	$\log_{10} N/N_o$ x 100
0 0	100	2.0000
1/2 1/2	111.0	2.0453
1 1	137.3	2.1377
1 1/2 1 1/2	233.4	2.3681

TABLE 37

Brain heart infusion agar

Incubation time (hours)	SET 1 BHI agar plate count	SET 1 Mean of three plate counts per disc	SET 1 N/N_o x 100 mean	SET 2 BHI agar plate count	SET 2 Mean of three plate counts per disc	SET 2 N/N_o x 100 mean
0	78, 80, 72	7.7×10^3	120.3	49, 44, 42	4.5×10^4	93.8
0	43, 63, 46	5.1×10^3	79.7	45, 60, 49	5.1×10^4	106.2
1/2				64, 70, 67	6.7×10^4	139.6
1/2				76, 65, 62	6.8×10^4	141.7
1	84, 87, 99	9.0×10^3	140.6	71, 82, 64	7.2×10^4	150.0
1	75, 88, 89	8.4×10^3	131.3	77, 67, 74	7.3×10^4	152.1
1 1/2				117, 138, 111	1.22×10^5	254.2
1 1/2				197, 152, 192	1.80×10^5	375.0
2	270, 277, 263	2.70×10^4	421.9	L.A.		
2	334, 359, 333	3.42×10^4	534.4	214, 247, 227	2.29×10^5	477.1

SUMMARY

	N/N_o x 100 mean of 2 sets	$\log_{10} N/N_o \times 100$
0	100	2.0000
0		
1/2	140.7	2.1483
1/2		
1	143.5	2.1569
1		
1 1/2	314.6	2.4978
1 1/2		
2	477.8	2.6793
2		

TABLE 38

Raw chicken meat

	SET 1			SET 2			Summary	
Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts per disc	N/N_o x 100 mean	BHI agar plate count	Mean of three plate counts per disc	N/N_o x 100 mean	N/N_o x 100 mean of two 2 sets	Log_{10} N/N_o x 100
0	45, 41, 46	4.4×10^4	91.7	73, 66, 72	7.0×10^4	96.6	100	2.0000
0	46, 53, 56	5.2×10^4	108.3	72, 78, 74	7.5×10^4	103.4		
1/2	59, 55, 65	6.0×10^4	125.0	76, 72, 72	7.3×10^4	100.7	114.4	2.0584
1/2	56, 64, 57	5.9×10^4	122.9	80, 73, 84	7.9×10^4	109.0		
1				91,100,104	9.8×10^5	135.2	122.4	2.0878
1				97,119, 86	1.01×10^5	139.3		
1 1/2	79, 59, 77	7.2×10^4	150.0	147,135,144	1.42×10^5	195.9	170.4	2.2315
1 1/2	86, 65, 81	7.7×10^4	160.4	122,133,127	1.27×10^5	175.2		
2	96, 97,103	9.9×10^5	206.3	127,140,107	1.25×10^5	172.4	204.7	2.3111
2	100, 95,116	1.04×10^5	216.7	152,184,151	1.62×10^5	223.5		

TABLE 39

Heat processed chicken meat

Incubation time (hrs.)	SET 1 BHI agar Plate count	SET 1 Mean of three plate counts per disc	SET 1 N/N_o x 100 mean	SET 2 BHI agar plate count	SET 2 Mean of three plate counts per disc	SET 2 N/N_o x 100 mean
0	99, 73, 90	8.7×10^3	108.1	86,117, 87	9.7×10^4	96.0
0	83, 62, 77	7.4×10^3	91.9	99,107,110	1.05×10^5	104.0
1/2	L. A.			L.A.102,111	1.07×10^5	105.9
1/2	84, 76, 87	8.2×10^3	101.9	99,102,104	1.02×10^5	101.0
1	91, 91, 82	8.8×10^3	109.3	146,146,152	1.48×10^5	146.5
1	107, 96, 94	9.9×10^4	123.0	128,138,143	1.36×10^5	134.7
1 1/2	113,107,130	1.13×10^4	140.4	124,129,120	1.24×10^5	122.8
1 1/2	92,113, 98	1.01×10^4	125.5	143,170,132	1.48×10^5	146.5
2	170,161,168	1.66×10^4	206.2	186,163,169	1.73×10^5	171.3
2	199,202,183	1.95×10	242.2	202,229,280	2.37×10^5	234.7

(continued)

TABLE 39

Heat processed chicken meat

(continued)

Incubation time (hrs.)	SET 3 BHI agar plate count	SET 3 Mean of three plate counts per disc	SET 3 N/N_o x 100 mean	SUMMARY N/N_ox100 mean of three sets	SUMMARY Log_{10} N/N_ox100
0	109,104, 91	$1.01x10^5$	99.5	100	2.0000
0	111, 94,100	$1.02x10^5$	100.5		
1/2	98,106, 75	$9.3 x10^4$	91.6	102.5	2.0107
1/2	127, 97,118	$1.14x10^5$	112.3		
1	132,125,124	$1.27x10^5$	125.1	126.3	2.1014
1	126,134,103	$1.21x10^5$	119.2		
1 1/2	148,157,158	$1.54x10^5$	151.7	135.0	2.1303
1 1/2	121,129,124	$1.25x10^5$	123.2		
2	192,153,178	$1.74x10^5$	171.4	205.5	2.3128
2	203,215,212	$2.10x10^5$	206.9		

TABLE 40

Irradiated chicken meat

Dose: $1x10^6$ rads of ionizing radiations*

Incubation time (hrs.)	SET 1 BHI agar plate count	SET 1 Mean of three plate counts Per disc	SET 1 $N/N_o x100$ mean	SET 2 BHI agar plate count	SET 2 Mean of three plate counts per disc	SET 2 $N/N_o x100$ mean
0	113,101, 95	$1.03x10^4$	101.5	91, 92, 81	$8.8 x10^3$	97.8
0	102,112, 86	$1.00x10^4$	98.5	83, 86,107	$9.2 x10^3$	102.2
1	109,101,115	$1.08x10^4$	106.4	94,105,118	$1.06x10^4$	117.8
1	102, 77, 95	$9.1 x10^3$	89.7	138,109,147	$1.31x10^4$	145.6
2	151,168,151	$1.57x10^4$	154.7	179,161,154	$1.65x10^4$	183.2
2	218,225,232	$2.25x10^4$	221.7	209,224,202	$2.12x10^4$	235.6
3	38, 47, 39	$4.1 x10^4$	403.9	239,241,202	$2.27x10^4$	252.2
3	264,254,274	$2.64x10^4$	260.1	162,180,161	$1.68x10^4$	186.7

SUMMARY

Incubation time (hrs.)	$N/N_o x100$ Mean of 2 sets	Log_{10} $N/N_o x100$
0 0	100	2.0000
1 1	114.9	2.0603
2 2	176.3	2.2463
3 3	275.7	2.4404

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 41

Irradiated chicken meat

Dose: 3×10^6 rads of ionizing radiations *

Incubation time (hrs.)	SET 1 BHI agar plate count	SET 1 Mean of three plate counts per disc	SET 1 $N/N_o \times 100$ mean	SET 2 BHI agar plate count	SET 2 Mean of three plate counts per disc	SET 2 $N/N_o \times 100$ mean
0	83, 71, 83	7.9×10^3	86.3	117, 97,107	1.07×10^4	97.3
0	117, 94,102	1.04×10^4	113.7	100,125,L.A.	1.13×10^4	102.7
1	102, 80, 97	9.3×10^3	101.6	119,113,117	1.16×10^4	105.5
1	87, 76, 93	8.5×10^3	92.9	111, 90, 96	9.9×10^3	90.0
2	106,103,99	1.03×10^4	112.6	124,129,140	1.31×10^4	119.1
2	99, 99, 90	9.6×10^3	104.9	157,165,158	1.60×10^4	145.5
3	114,106,122	1.14×10^4	124.6	249,219,184	2.17×10^4	197.3
3	96, 92, 96	9.5×10^3	103.8	163,202,187	1.84×10^4	167.3
4	119,143,140	1.34×10^4	146.4	221,231,225	2.26×10^4	205.5
4	112,122,109	1.14×10^4	124.6	284,293,302	2.93×10^4	266.4
5	145,146,133	1.41×10^4	154.1	277,308,320	3.02×10^4	274.6
5	158,183,173	1.71×10^4	186.9	346,362,356	3.55×10^4	322.7
6	204,220,232	2.19×10^4	239.3	61, 73, 44	5.9×10^4	536.4
6	255,232,241	2.43×10	265.6	35, 41, 48	4.1×10	372.7

(continued)

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 41

Irradiated chicken meat

Dose: 3×10^6 rads of ionizing radiations

(continued)

SUMMARY

Incubation time (hrs.)	$N/N_o \times 100$ mean of 2 sets	Log_{10} $N/N_o \times 100$
0 0	100	2.0000
1 1	97.5	1.9890
2 2	120.5	2.0810
3 3	148.3	2.1711
4 4	185.7	2.2686
5 5	234.6	2.3603
6 6	353.5	2.5484

TABLE 42

Irradiated chicken meat

Dose: 6×10^6 rads of Ionizing radiations*

Incubation time (hrs.)	SET 1 BHI agar plate count	SET 1 Mean of three plate counts per disc	SET 1 N/N_o mean x 100	SET 2 BHI agar plate count	SET 2 Mean of three plate counts per disc	SET 2 N/N_o mean x 100
0	82, 80, 88	8.3×10^3	100	95, 74, 99	8.9×10^3	95.2
0	82, 86, 81	8.3×10^3	100	97, 98, 98	9.8×10^3	104.8
1	74, 81, 86	8.0×10^3	96.4	69, 73, 70	7.1×10^3	75.9
1	75, 76, 84	7.8×10^3	94.0	88, 97, 98	9.4×10^3	100.5
2	70, 69, 70	7.0×10^3	84.3	82, 69, 82	7.8×10^3	83.4
2	78, 77, 86	8.0×10^3	96.4	66, 76, 78	7.3×10^4	78.1
3	85, 85, 80	8.3×10^4	100	110,120,142	1.24×10^3	132.6
3	100,102,107	1.03×10^4	124.1	98, 77, 99	9.1×10^3	97.3
4	120,130, 91	1.14×10^3	137.4	154,123,172	1.50×10^4	160.4
4	60, 72, 81	7.1×10^4	85.5	126,135,134	1.32×10^4	141.1
5	119,116,118	1.18×10^4	142.2	113,118,147	1.26×10^4	134.8
5	136,137,121	1.31×10^4	157.8	175,167,179	1.74×10^4	186.1
6	139,137,144	1.40×10^4	168.7	173,171,194	1.79×10^4	191.4
6	175,148,150	1.58×10^4	190.4	213,168,164	1.82×10^4	194.7
7	144,144,168	1.52×10^4	183.1	171,161,165	1.66×10^4	177.5
7	131,113,115	1.20×10^4	144.6	191,153,154	1.66×10^4	177.5
8	128,146,137	1.37×10^4	165.1	13, 18, 22	1.8×10^4	192.5
8	147,158,175	1.60×10^4	192.8	15, 16, 14	1.5×10^4	160.4
9	229,226,216	2.24×10^4	269.9	21, 22, 20	2.1×10^4	224.6
9	148,152,143	1.48×10	178.3	39, 25, 18	2.7×10	288.8

(continued)

*Ionizing radiations produced by a 2 Mev Van de Graaf accelerator.

TABLE 42

Irradiated chicken meat

Dose: 6×10^6 rads of ionizing radiations

(continued)

SUMMARY

Incubation time (hrs.)	$N/N_o \times 100$ Mean of 2 sets	Log_{10} $N/N_o \times 100$
0 0	100	2.0000
1 1	91.7	1.9624
2 2	85.6	1.9320
3 3	113.5	2.0550
4 4	131.1	2.1173
5 5	155.2	2.1909
6 6	186.3	2.2702
7 7	170.7	2.2322
8 8	177.7	2.2497
9 9	240.4	2.3809

F. Data accumulated during the logarithmic phase of growth of Staphylococcus aureus at 37°C in and on various media

TABLE 43

Brain heart infusion

(at rest)

Set no.	Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts per ml.	Log_{10} mean
1	1 1/2	154, 166, 154	1.58×10^5	5.1987
	1 1/2	193, 169, 189	1.84×10^5	5.2648
	2	37, 27, 32	3.2×10^5	5.5051
	2	46, 55, 50	5.0×10^5	5.6990
	3	154, 127, 127	1.36×10^6	6.1335
	3	133, 153, 159	1.48×10^6	6.1703
	5	124, 121, 125	1.23×10^7	7.0899
	5	38, 37, 41	3.9×10^7	7.5911
	6	232, 255, 269	2.52×10^8	8.4014
	6	175, 188, 192	1.85×10^8	8.2672
	7	42, 45, 41	4.3×10^8	8.6335
	7	166, 147, 150	1.54×10^8	8.1875
	8	76, 68, 62	6.9×10^8	8.8388
	8	103, 82, 75	8.7×10^8	8.9395
2	1 1/2	158, 110, 133	1.34×10^5	5.1271
	1 1/2	167, 195, 143	1.68×10^5	5.2253
	2	24, 28, 46	3.3×10^5	5.5185
	2	35, 50, 32	3.9×10^5	5.5911
	3	136, 124, 135	1.32×10^6	6.1206
	3	150, 173, 166	1.63×10^6	6.2122
	4	77, 101, 73	8.4×10^6	6.9243
	4	79, 65, 94	7.9×10^6	6.8976
	5	35, 39, 35	3.6×10^7	7.5563
	5	35, 47, 36	3.9×10^7	7.5911
	6	155, 177, 162	1.65×10^8	8.2175
	6	209, 208, 225	2.14×10^8	8.3304
	7	60, 48, 38	6.5×10^8	8.6902
	7	55, 60, 51	6.2×10^8	8.7407
3	1 1/2	240, 228, 194	2.21×10^5	5.3444
	1 1/2	235, 215, -	2.25×10^5	5.3522
	2	51, 41, 47	4.6×10^5	5.6628
	2	44, 45, 48	4.5×10^5	5.6532
	2 1/2	80, 79, 75	7.8×10^5	5.8921
	2 1/2	89, 105, 95	9.8×10^5	5.9912
	3	192, 218, 218	2.09×10^6	6.3201
	3	211, 188, 189	1.96×10^6	6.2923
	3 1/2	64, 52, 46	5.4×10^6	6.7324
	3 1/2	58, 51, 32	4.7×10^6	6.6721
	4	102, 103, 102	1.02×10^7	7.0086
	4	153, 130, 144	1.42×10^7	7.1523
	5	51, 55, 54	5.3×10^7	7.7243
	5	67, 58, 51	5.9×10^7	7.7709
	6	206, 194, 191	1.97×10^8	8.2945
	6	153, 152, 140	1.48×10^8	8.1703
	7	37, 32, 33	3.4×10^8	8.5315
	7	41, 38, 32	3.7×10^8	8.5682

TABLE 44

Brain heart infusion

(with agitation)

Set no.	Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts per ml.	Log_{10} mean
1	2 1/2	35, 40, 40	3.8×10^5	5.5798
	3	97, 125, 90	1.04×10^6	6.0170
	4	64, 69, 74	6.9×10^7	6.8388
	5	308, 308, 279	2.98×10^8	7.4742
	6	163, 141, 165	1.56×10^8	8.1931
	7	75, 80, 63	7.3×10^9	8.8633
	8	375, 286, 340	3.34×10^9	9.5237
2	2 1/2	65, 79, 80	7.5×10^5	5.8751
	3	175, 178, 161	1.71×10^6	6.2330
	4	96, 96, 96	9.6×10^7	6.9823
	5	47, 50, 40	4.6×10^8	7.6628
	6	214, 225, 223	2.21×10^8	8.3444
	7	102, 91, 83	9.2×10^8	8.9638

TABLE 45

Brain heart infusion agar

Set no.	Incubation time (hours)	BHI agar plate count	Mean of three plate counts per disc	Log_{10} mean
1	2	117, 131, 109	1.19×10^5	5.0755
	2	216, 189, 228	2.11×10^5	5.3243
	2 1/2	51, 55, 51	5.2×10^5	5.7160
	2 1/2	39, 45, 43	4.2×10^5	5.6232
	3	87, 62, 77	7.5×10^5	5.8751
	3	91, 69, 59	7.3×10^5	5.8633
	4	323, 340, 317	3.27×10^6	6.5145
	4	262, 253, 269	2.61×10^6	6.4166
	5	95, 90, 99	9.5×10^7	6.9777
	5	142, 130, 137	1.36×10^7	7.1335
	6	61, 55, 65	6.0×10^7	7.7782
	6	112, 93, 100	1.02×10^8	8.0086
	7	26, 26, 44	3.2×10^8	8.5051
	7	61, 56, 67	6.1×10^5	8.7853
2	1 1/2	117, 138, 111	1.22×10^5	5.0864
	1 1/2	197, 152, 192	1.80×10^5	5.2553
	2 1/2	68, 51, 74	6.4×10^5	5.8062
	2 1/2	65, 67, 58	6.3×10^5	5.7993
	3	134, 127, 120	1.27×10^6	6.1038
	3	141, 117, 136	1.31×10^6	6.1173
	4	43, 59, 67	5.6×10^6	6.7482
	4	79, 48, 85	7.1×10^7	6.8513
	5	190, 199, 189	1.93×10^7	7.2856
	5	273, 254, 262	2.63×10^8	7.4200
	6	115, 123, 87	1.08×10^8	8.0334
	6	115, 115, 87	1.06×10^8	8.0253
	7	54, 33, 39	4.2×10^8	8.6232
	7	51, 52, 57	5.3×10^8	8.7243
3	2	270, 277, 263	2.70×10^4	4.4314
	2	334, 359, 333	3.4×10^5	4.5340
	3	111, 109, 124	1.15×10^5	5.0607
	3	112, 120, 108	1.13×10^5	5.0531
	4	44, 54, 47	4.8×10^5	5.6812
	4	81, 55, 53	6.3×10^5	5.7993
	5	179, 212, 205	1.99×10^6	6.2989
	5	321, 295, 272	2.96×10^6	6.4713
	6	122, 125, 125	1.24×10^7	7.0934
	6	139, 113, 110	1.21×10^7	7.0828
	7	59, 70, 85	7.1×10^7	7.8513
	7	74, 59, 71	6.8×10^7	7.8325
	8	50, 42, 30	4.1×10^8	8.6128
	8	47, 38, 46	4.4×10^8	8.6435
	9	113, 117, 132	1.21×10^9	9.0828
	9	210, 195, 209	2.05×10^9	9.3118

TABLE 46

Raw chicken meat

Set no.	Incubation time (hrs.)	BHI agar Plate count	Mean of three plate counts per disc	Log_{10} mean
1	4	50, 46, 44	4.7×10^5	5.6721
	4	35, 42, 39	3.9×10^5	5.5911
	5	80, 84, 102	8.9×10^5	5.9494
	5	260, 281, 263	2.68×10^6	6.4281
	6	56, 51, 47	5.1×10^6	6.7076
	6	317, 314, 286	3.06×10^7	6.4857
	7	103, 101, 114	1.06×10^7	7.0253
	7	121, 125, 157	1.34×10^7	7.1271
	8	52, 61, 61	5.8×10^7	7.7634
	8	99, 92, 111	1.01×10^8	8.0043
	9	144, 191, 153	1.63×10^8	8.2122
	9	150, 156, 123	1.43×10^8	8.1553
2	3	36, 31, 33	3.3×10^5	5.5185
	3	33, 32, 32	3.2×10^5	5.5051
	4	35, 36, 35	3.5×10^5	5.5441
	4	96, 121, 95	1.04×10^6	6.0170
	5	122, 115, 128	1.22×10^6	6.0864
	5	87, 87, 91	8.8×10^5	5.9445
	6	65, 72, 78	7.2×10^6	6.8573
	6	98, 77, 97	9.1×10^6	6.9590
	8	86, 82, 100	8.9×10^7	7.9494
	8	33, 24, 37	3.1×10^7	7.4914
3	3 1/2	37, 39, 35	3.7×10^5	5.5682
	3 1/2	50, 55, 34	4.6×10^5	5.6628
	4	39, 51, 49	4.6×10^5	5.6628
	4	102, 86, 86	9.1×10^5	5.9590
	5	137, 132, 132	1.34×10^6	6.1271
	5	142, 159, 134	1.45×10^6	6.1614
	6	104, 107, 115	1.09×10^7	7.0374
	6	79, 66, 74	7.3×10^6	6.8633
	7	246, 280, 259	2.62×10^7	7.4183
	7	125, 118, 118	1.20×10^7	7.0792
	8	40, 43, 45	4.3×10^7	7.6335
	8	284, 271, 274	2.76×10^8	7.4409
	9	111, 125, 110	1.15×10^7	8.0607
	9	98, 93, 96	9.6×10^7	7.9823

TABLE 47

Heat processed chicken meat
Initial slope

Set no.	Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts per disc	Log_{10} mean
1	2 1/2	176, 151, 197	1.75×10^5	5.2430
	2 1/2	42, 28, 36	3.5×10^5	5.5441
	3	63, 56, 80	6.6×10^5	5.8195
	3	82, 78, 76	7.9×10^5	5.8976
	4	91, 99, 87	9.2×10^5	5.9638
	4	80, 111, 95	9.5×10^7	5.9777
	6	21, 27, 22	2.3×10^7	7.3617
	6	27, 23, 17	2.2×10^7	7.3424
2	2 1/2	29, 34, 32	3.2×10^5	5.5051
	2 1/2	35, 33, 23	3.0×10^5	5.4771
	3	44, 54, 50	4.9×10^5	5.6902
	3	72, 50, 59	6.0×10^5	5.7782
	4	186, 201, 207	1.98×10^6	6.2967
	4	343, 367, 360	3.57×10^7	6.5527
	5	104, 98, 108	1.03×10^7	7.0128
	5	143, 150, 147	1.47×10^7	7.1673
3	2 1/2	190, 223, 196	1.99×10^4	4.2989
	2 1/2	227, 234, 208	2.23×10^4	4.3483
	3	35, 34, 28	3.2×10^4	4.5051
	3	29, 36, 34	3.3×10^4	4.5185
	4	74, 64, 77	7.2×10^5	4.8573
	4	124, 111, 131	1.22×10^5	5.0864
	5	27, 40, 40	3.6×10^5	5.5563
	5	34, 42, 49	4.2×10^5	5.6232
	6	261, 339, 317	3.06×10^6	6.4857
	6	207, 235, 236	2.26×10^6	6.3541
	7	50, 64, 41	5.2×10^6	6.7160
	7	102, 96, 80	9.3×10^6	6.9685

TABLE 48

Heat processed chicken meat
Secondary slope

Set no.	Incubation time (hrs.)	BHI agar plate counts	Mean of three plate counts per disc	Log_{10} mean
1	5	104, 98, 108	1.03×10^7	7.0128
	5	143, 150, 147	1.47×10^7	7.1673
	6	38, 42, 51	4.4×10^7	7.6435
	6	85, 73, 98	8.5×10^6	6.9294
	7	50, 57, 48	5.2×10^7	7.7160
	7	63, 54, 47	5.5×10^7	7.7404
	8	83, 90, 105	9.3×10^7	7.9685
	8	133, 120, 156	1.36×10^8	8.1335
	9	180, 161, 216	1.86×10^8	8.2695
	9	25, 27, 38	3.0×10^8	8.4771
2	7	50, 64, 41	5.2×10^6	6.7160
	7	102, 96, 80	9.3×10^7	6.9685
	8	150, 128, 152	1.43×10^6	7.1553
	8	109, 85, 92	9.5×10^7	6.9777
	9	31, 39, 44	3.8×10^7	7.5798
	9	145, 171, 157	1.58×10^7	7.1987

TABLE 49

Irradiated chicken meat

Dose: 1×10^6 rads of ionizing radiations*

Set no.	Incubation time (hrs.)	BHI agar Plate count	Mean of three plate counts per disc	Log_{10} mean
1	4	51, 42, 52	4.8×10^4	4.6812
	4	85, 79, 84	8.3×10^4	4.9191
	5	50, 57, 39	4.9×10^5	4.6902
	5	117, 110, 138	1.22×10^5	5.0864
	6	47, 55, 48	5.0×10^5	5.6990
	6	35, 31, 26	3.1×10^5	5.4914
	7	63, 65, 63	6.4×10^5	5.8062
	7	274, 244, 242	2.53×10^6	6.4031
	8	42, 19, 41	3.4×10^6	6.5315
	8	94, 105, 116	1.05×10^7	7.0212
	9	98, 107, 101	1.02×10^7	7.0086
	9	81, 67, 85	7.8×10^6	6.8921
	10	187, 186, 184	1.86×10^7	7.2695
	10	144, 161, 151	1.52×10^7	7.1818
	11	37, 33, 29	3.6×10^7	7.5563
	11	68, 60, 55	6.8×10^7	7.8325
2	4	49, 46, 59	5.1×10^4	4.7076
	4	48, 42, 30	4.0×10^5	4.6021
	5	119, 105, 140	1.21×10^5	5.0828
	5	71, 87, 84	8.1×10^4	4.9085
	6	102, 126, 125	1.18×10^6	6.0719
	6	47, 49, 52	4.9×10^5	5.6902
	7	37, 28, 31	3.2×10^5	5.5051
	7	160, 154, 198	1.71×10^6	6.2330
	8	189, 177, 202	1.89×10^6	6.2765
	8	87, 80, 71	7.9×10^6	6.8976
	9	48, 59, 60	5.6×10^6	6.7482
	9	294, 294, 281	2.90×10^7	7.4624
	10	172, 193, 181	1.82×10^7	7.2601
	10	143, 121, 103	1.22×10^7	7.0864
	11	40, 21, 41	3.4×10^7	7.5315
	11	126, 146, 143	1.38×10^8	8.1399

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 50

Irradiated chicken meat
Dose: 3×10^6 rads of ionizing radiations*

Set no.	Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts per disc	Log_{10} mean
1	18	37, 26, 39	3.4×10^6	6.5315
	18	26, 28, 35	3.0×10^6	6.4771
	19	86, 59, 59	6.8×10^7	6.8325
	19	180, 173, 165	1.73×10^7	7.2380
	20	204, 178, 166	1.83×10^7	7.2625
	20	85, 72, 82	8.0×10^6	6.9031
	22	30, 28, 36	3.1×10^7	7.4914
	22	37, 45, 33	3.8×10^7	7.5798
2	20	54, 61, 66	6.0×10^6	6.7782
	20	59, 36, 43	4.6×10^6	6.6628
	21	63, 80, 58	6.7×10^7	6.8261
	21	132, 125, 136	1.31×10^7	7.1173
	22	138, 167, 155	1.53×10^7	7.1847
	22	171, 150, 166	1.62×10^7	7.2095
3	12	135, 116, 129	1.27×10^5	5.1038
	12	29, 22, 35	2.9×10^5	5.4624
	13	46, 51, 59	5.2×10^5	5.7160
	13	125, 130, 137	1.31×10^6	6.1173
	14	61, 54, 61	5.9×10^5	5.7709
	14	117, 124, 133	1.25×10^6	6.0969
	15	287, 289, 275	2.84×10^6	6.4533
	15	41, 63, 59	5.4×10^6	6.7324
	17	31, 35, 30	3.2×10^6	6.5051
	17	52, 52, 42	4.9×10^6	6.6902
	18	237, 286, 275	2.66×10^6	6.4249
	18	48, 59, 49	5.2×10^6	6.7160
	19	77, 77, 80	7.8×10^7	6.8921
	19	101, 137, 159	1.32×10^7	7.1206
	20	190, 189, 219	1.99×10^7	7.2989
	20	74, 62, 71	6.9×10^6	6.8388
	21	208, 186, 214	2.03×10^7	7.3075
	21	36, 32, 33	3.4×10^7	7.5315

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 51

Irradiated chicken meat

Dose: 6×10^6 rads of ionizing radiations*

Set no.	Incubation time (hrs.)	BHI agar Plate count	Mean of three plate counts per disc	Log_{10} mean
1	8	128, 146, 137	1.37×10^4	4.1367
	8	147, 158, 175	1.60×10^4	4.2041
	9	229, 226, 216	2.24×10^4	4.3502
	9	148, 152, 143	1.48×10^4	4.1703
	10	285, 309, 268	2.87×10^4	4.4579
	10	245, 230, 230	2.35×10^4	4.3711
	11	324, 278, 331	3.11×10^4	4.4928
	11	34, 39, 33	3.5×10^4	4.5441
	12	35, 54, 59	4.9×10^4	4.6902
	12	43, 44, 42	4.3×10^4	4.6335
	21	62, 49, 50	5.4×10^6	6.7324
	21	21, 22, 9	1.7×10^6	6.2304
	22	57, 61, 51	5.6×10^6	6.7482
	22	86, 73, 67	7.5×10^6	6.8751
	23	101, 107, 88	9.9×10^6	6.9956
	23	105, 104, 90	1.00×10^7	7.0000
	24	159, 167, 186	1.71×10^7	7.2330
	24	121, 87, 98	1.02×10^7	7.0086
	25	207, 207, 193	2.02×10^7	7.3054
	25	235, 303, 253	2.64×10^7	7.4216
	26	24, 32, 42	3.3×10^7	7.5185
	26	37, 18, 33	2.9×10^7	7.4624
2	12	50, 60, 73	6.1×10^4	4.7853
	12	35, 35, 42	3.7×10^4	4.5682
	13	78, 70, 45	6.4×10^4	4.8062
	13	92, 87, 91	9.0×10^4	4.9542
	16	32, 36, 33	3.4×10^5	5.5315
	16	150, 127, 122	1.33×10^5	5.1239
	17	34, 38, 38	3.7×10^5	5.5682
	17	75, 75, 75	7.5×10^5	5.8751
	18	76, 71, 71	7.3×10^5	5.8633
	18	102, 74, 84	8.6×10^5	5.9345
	19	181, 179, 162	1.74×10^6	6.2405
	19	82, 97, 79	8.6×10^5	5.9345
	20	173, 197, 183	1.84×10^6	6.2648
	20	151, 173, 138	1.54×10^6	6.1875
	21	33, 40, 49	4.1×10^6	6.6128
	21	21, 35, 27	2.8×10^6	6.4472

(continued)

TABLE 51

Irradiated chicken meat
Dose: 6×10^6 rad of ionizing radiations
(continued)

Set no.	Incubation time (hrs.)	BHI agar Plate count	Mean of three plate counts per disc	Log_{10} mean
3	12	209, 203, 261	2.24×10^4	4.3502
	12	75, 55, 80	7.0×10^3	3.8451
	13	56, 58, 67	6.0×10^4	4.7782
	13	313, 287, 255	2.85×10^4	4.4548
	14	14, 16, 27	1.9×10^4	4.2788
	14	41, 49, 45	4.5×10^4	4.6532
	15	51, 47, 39	4.6×10^4	4.6628
	15	34, 26, 35	3.2×10^4	4.5051
	16	47, 53, 60	5.3×10^4	4.7243
	16	40, 41, 54	4.5×10^4	4.6532
	17	82, 110, 97	9.6×10^5	4.9823
	17	123, 126, 146	1.32×10^5	5.1206
	18	54, 64, 46	5.5×10^5	5.7404
	18	24, 19, 20	2.1×10^5	5.3222
	20	26, 27, 25	2.6×10^5	5.4150
	20	56, 72, 48	5.9×10^5	5.7709
	21	49, 46, 49	4.8×10^6	5.6812
	21	93, 136, 155	1.28×10^6	6.1072
	22	37, 46, 51	4.5×10^6	6.6532
	22	161, 170, 166	1.66×10^6	6.2201
	24	43, 33, 44	4.0×10^6	6.6021
	24	18, 19, 19	1.9×10^7	6.2788
	27	136, 118, 93	1.16×10^7	7.0645
	27	37, 48, 42	4.2×10^7	7.6232
	28	236, 212, 221	2.23×10^7	7.3483
	28	211, 190, 207	2.03×10^7	7.3075

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

G. Data accumulated during the stationary phase of Staphylcoccus aureus at 37°C on and in various media

TABLE 52

Brain heart infusion (at rest)

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 1		
24	33, 48, 32	3.8×10^9	4.0×10^9	9.6021
24	37, 51, 38	4.2×10^9		
27	42, 31, 40	3.8×10^9	3.3×10^9	9.5185
27	19, 30, 35	2.8×10^9		
28	21, 35, 31	2.9×10^9	3.1×10^9	9.4914
28	29, 28, 41	3.3×10^9		
30	35, 34, 34	3.4×10^9	3.1×10^9	9.4914
30	30, 23, 31	2.8×10^9		
31				
31				
32				
32				
33				
33				
35	33, 37, 43	3.8×10^9	3.3×10^9	9.5185
35	24, 35, 26	2.8×10^9		
		SET 2		
24				
24				
27				
27				
28				
28				
30	31, 33, L.A.	3.2×10^9	3.3×10^9	9.5185
30	31, 41, 29	3.4×10^9		
31	282,282,286	2.83×10^9	3.0×10^9	9.4771
31	39, 25, 32	3.2×10^9		
32	22, 33, 32	2.9×10^9	3.2×10^9	9.5051
32	33, 30, 40	3.4×10^9		
33	32, 28, 29	3.0×10^9	3.0×10^9	9.4771
33	282,320,297	3.00×10^9		
35				
35				

TABLE 53

Brain heart infusion agar

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 1		
13	221,223,240	2.28×10^{10}		
13	241,250,251	2.47×10^{10}	2.38×10^{10}	10.3766
21	71, 80, 83	7.8×10^{10}		
21	39, 53, 54	4.9×10^{10}	6.4×10^{10}	10.8062
27	38, 55, 42	4.5×10^{10}		
27	34, 40, 47	4.0×10^{10}	4.3×10^{10}	10.6335
28	51, 50, 55	5.2×10^{10}		
28	44, 56, 43	4.8×10^{10}	5.0×10^{10}	10.6990
29	29, 50, 42	4.0×10^{10}		
29	46, 50, 64	5.3×10^{10}	4.7×10^{10}	10.6721
31	46, 38, 34	3.9×10^{10}		
31	42, 25, 25	3.1×10^{10}	3.5×10^{10}	10.5441
32				
32				
33	30, 37, 31	3.3×10^{10}		
33	28, 21, 33	2.7×10^{10}	3.0×10^{10}	10.4771
47	155,128,143	1.42×10^{10}		
47	128,141,132	1.34×10^{10}	1.38×10^{10}	10.1399
54	141,143,151	1.45×10^{10}		
54	119,141,126	1.29×10^{10}	1.37×10^{10}	10.1367
		SET 2		
13				
13				
21				
21				
27				
27				
28	42. 37, 44	4.1×10^{10}		
28	49, 45, 58	5.1×10^{10}	4.6×10^{10}	10.6628
29				
29				
31				
31				
32	37, 35, 32	3.5×10^{10}		
32	40, 24, 38	3.4×10^{10}	3.5×10^{10}	10.5441
33				
33				
47				
47				
54				
54				

TABLE 54

Raw chicken meat

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 1		
12				
12				
13				
13				
14				
14				
15				
15				
16				
16				
17				
17				
18				
18				
19				
19				
24	180,181,206	1.89×10^9		
24	168,127,147	1.47×10^9	1.68×10^9	
25				
25				
26	141,154,181	1.59×10^9		
26	103,105, 88	9.9×10^8	1.29×10^9	
27				
27				
28	243,223,258	2.41×10^9		
28	277,229,239	2.48×10^9	2.45×10^9	
29	Lab. accident			
29	139,141,161	1.47×10^9	1.47×10^9	
30	118,129,122	1.23×10^9		
30	129,135,131	1.32×10^9	1.28×10^9	
31				
31				
33				
33				
34				
34				
35				
35				
36				
36				
47				
47				
49				
49				
52				
52		(continued)		

TABLE 54

Raw chicken meat

(continued)

Incubation time (hrs.	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 2		
12				
12				
13				
13				
14				
14				
15				
15				
16				
16				
17				
17				
18				
18				
19				
19				
24				
24				
25				
25				
26				
26				
27				
27				
28	68, 73, 68	7.0×10^8		
28	215,240,241	2.32×10^9	1.5×10^9	9.1761
29				
29				
30				
30				
31	131,120,114	1.22×10^9		
31	184,166,170	1.73×10^9	1.50×10^9	9.1761
33				
33				
34				
34				
35				
35				
36				
36				
47				
47				
49				
49				
52				
52				

(continued)

Raw chicken meat
(continued)

TABLE 54

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 3		
12				
12				
13				
13				
14				
14				
15				
15				
16				
16				
17				
17				
18				
18				
19				
19				
24				
24				
25				
25				
26				
26				
27				
27				
28				
28				
29				
29				
30				
30				
31				
31				
33	139,103,129	1.24×10^9	1.23×10^9	9.0899
33	125,122,118	1.22×10^9		
34	132,141,129	1.34×10^9	1.26×10^9	9.1004
34	110,123,120	1.18×10^9		
35				
35				
36	170,160,166	1.65×10^9	1.67×10^9	9.2227
36	180,152,175	1.69×10^9		
47				
47				
49				
49				
52				
52				

(continued)

TABLE 54

Raw chicken meat

(continued)

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 4		
12	57, 41, 49	4.9×10^8	4.5×10^8	8.6532
12	28, 46, 50	4.1×10^8		
13	46, 38, 48	4.4×10^8	4.9×10^8	8.6902
13	53, 60, 47	5.3×10^8		
14	34, 35, 31	3.3×10^8	5.6×10^8	8.7482
14	82, 67, 88	7.9×10^8		
15	47, 47, 51	4.8×10^8	5.6×10^8	8.7482
15	68, 65, 60	6.4×10^9		
16	186,165,174	1.75×10^8	1.33×10^9	9.1239
16	86,103, 81	9.0×10^9		
17	112,124, 91	1.09×10^9	1.15×10^9	9.0607
17	124,101,138	1.21×10^9		
18	218,218,211	2.16×10^9	1.77×10^9	9.2480
18	137,124,153	1.38×10^9		
19	129,129,134	1.31×10^9	1.27×10^9	9.1038
19	128,110,129	1.22×10^9		
24				
24				
25				
25				
26				
26				
27				
27				
28				
28				
29				
29				
30				
30				
31				
31				
33				
33				
34				
34				
35				
35				
36				
36				
47				
47				
49				
49				
52				
52				

(continued)

TABLE 54 — Raw chicken meat (continued)

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 5		
12				
12				
13				
13				
14				
14				
15				
15				
16				
16				
17				
17				
18				
18				
19				
19				
24	57, 36, 47	4.6×10^8		
24	23, 25, 28	2.5×10^8	3.6×10^8	8.5563
25	54, 50, 50	5.1×10^8		
25	16, 16, 16	1.6×10^8	3.4×10^8	8.5315
26	21, 26, 32	2.6×10^8		
26	31, 27, 17	2.5×10^8	2.6×10^8	8.4150
27	41, 47, 41	4.3×10^8		
27	41, 40, 41	4.1×10^8	4.2×10^8	8.6232
28	102, 96, 119	1.06×10^9		
28	96, 103, 94	9.8×10^8	1.02×10^9	9.0086
29	30, 34, 19	2.8×10^8		
29	34, 31, 40	3.5×10^8	3.2×10^8	8.5051
30				
30				
31	40, 35, 43	3.9×10^8		
31	53, 71, 70	6.5×10^8	5.2×10^8	8.7160
33	34, 38, 37	3.6×10^8		
33	92, 71, 60	7.4×10^8	5.5×10^8	8.7404
34				
34				
35	81, 91, 75	8.2×10^8		
35	65, 60, 70	6.5×10^8	7.4×10^8	8.8692
36				
36				
47	125, 132, 118	1.25×10^9		
47	94, 81, 95	9.0×10^8	1.08×10^9	9.0334
49	Lab. accident			
49	206, 177, 199	1.94×10^9	1.94×10^9	9.2878
52	195, 186, 180	1.87×10^9		
52	57, 62, 52	5.7×10^8	1.21×10^9	9.0828

TABLE 55

Heat processed chicken meat

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 1		
9	180,161,216	1.86×10^8	2.43×10^8	8.3856
9	25, 27, 38	3.0×10^8		
24	34, 19, 28	2.7×10^8	2.7×10^8	8.4314
24	Lab. accident			
25	24, 24, 27	2.5×10^8	3.5×10^8	8.5441
25	52, 46, 35	4.4×10^8		
26	36, 37, 37	3.7×10^8	3.5×10^8	8.5441
26	39, 26, 30	3.2×10^8		
27	Lab. accident		3.4×10^8	8.5315
27	38, 33, 32	3.4×10^8		
28	62, 60, 47	5.6×10^8	4.2×10^8	8.6232
28	31, 26, 25	2.7×10^8		
30				
30				
		SET 2		
9				
9				
24				
24				
25				
25				
26	237,252,299	2.63×10^8	2.18×10^8	8.3385
26	168,192,158	1.73×10^8		
27				
27				
28				
28				
30	279,298,274	2.84×10^8	3.73×10^8	8.5717
30	465,500,424	4.63×10^8		

TABLE 56

Irradiated chicken meat

Dose: 1×10^6 rads of ionizing radiations*

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
11	37, 33, 29	3.6×10^7	5.2×10^7	7.7160
11	68, 80, 55	6.8×10^7		
12				
12				
13				
13				
20				
20				
21	141,129,112	1.27×10^8	1.02×10^8	8.0086
21	74, 72, 84	7.6×10^7		
22	39, 42, 38	4.0×10^7	4.0×10^7	7.6021
22	40, 39, 42	4.0×10^7		
23	58, 78, 53	6.3×10^7	6.0×10^7	7.7782
23	49, 66, 57	5.7×10^7		
24	59, 58, 63	6.0×10^7	7.3×10^7	7.8633
24	88, 77, 89	8.5×10^7		
25	68, 52, 58	6.1×10^7	6.0×10^7	7.7782
25	57, 54, 65	5.9×10^7		
26				
26				
27				
27				
28				
28				
31	100,103,117	1.07×10^8	2.79×10^8	8.4456
31	42, 48, 45	4.5×10^8		
45	76, 75, 86	7.9×10^8	6.7×10^8	8.8261
45	53, 50, 63	5.5×10^8		
47	37, 38, 39	3.8×10^9	9.0×10^8	8.9542
47	144,141,141	1.42×10^9		
49	305,251,321	2.92×10^9	2.50×10^9	9.3979
49	205,201,214	2.07×10^8		
51	57, 50, 58	5.5×10^9	8.3×10^8	9.1239
51	112,107,110	1.10×10^8		
53	57, 37, 46	4.7×10^9	1.05×10^9	9.1903
53	168,154,167	1.63×10^9		

(continued)

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 56

Irradiated chicken meat

Dose: 1×10^6 rad of ionizing radiations

(continued)

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 2		
11	40, 21, 41	3.4×10^7	8.6×10^7	7.9345
11	126,146,143	1.38×10^8		
12	246,248,262	2.52×10^8	1.52×10^8	8.1818
12	52, 42, 61	5.2×10^7		
13	65, 63, 63	6.4×10^7	6.8×10^7	7.8325
13	70, 64, 78	7.1×10^7		
20	33, 38, 29	3.3×10^7	1.90×10^8	8.2788
20	348,385,308	3.47×10^8		
21	234,272,267	2.58×10^8	1.71×10^8	8.2330
21	86, 83, 83	8.4×10^7		
22	120,101,110	1.10×10^7	2.15×10^7	7.3324
22	35, 32, 30	3.2×10^7		
23	26, 40, 31	3.2×10^7	3.2×10^7	7.5051
23	contaminated			
24	5x138,5x119,5x150	6.78×10^8	5.08×10^8	8.7059
24	329,343,339	3.37×10^8		
25				
25				
26	56, 55, 49	5.3×10^7	5.9×10^7	7.7709
26	64, 68, 64	6.5×10^7		
27	38, 42, 30	3.7×10^7	3.12×10^8	8.4942
27	253,257,253	2.54×10^8		
28	50, 48, 43	4.7×10^8	3.36×10^8	8.5263
28	184,192,230	2.02×10^8		
31				
31				
45				
45				
47				
47				
49				
49				
51				
51				
53				
53				

TABLE 57

Irradiated chicken meat

Dose: 3×10^6 rads of ionizing radiations*

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} Mean
		SET 1		
21				
21				
22	30, 28, 36	3.1×10^7	3.5×10^7	7.5441
22	37, 45, 33	3.8×10^7		
23	49, 54, 55	5.3×10^7	3.6×10^7	7.5563
23	172,184,180	1.79×10^7		
24	28, 35, 37	3.3×10^7	3.3×10^7	7.5185
24	38, 32, 27	3.2×10^7		
25	171,165,187	1.74×10^7	3.4×10^7	7.5315
25	57, 47, 47	5.0×10^7		
26	47, 38, 48	4.4×10^7	3.7×10^7	7.5682
26	28, 32, 28	2.9×10^7		
27				
27				
28				
28				
41				
41				
43				
43				
44				
44				
47	220,198,207	2.08×10^8	2.26×10^8	8.3541
47	243,230,259	2.44×10^8		
48	236,316,250	2.67×10^8	3.19×10^8	8.5038
48	45, 32, 34	3.7×10^8		
49	47, 53, 46	4.9×10^8	4.1×10^8	8.6128
49	331,300,351	3.27×10^8		

(continued)

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 57

Irradiated chicken meat

Dose: 3×10^6 rads of ionizing radiations

(continued)

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 2		
21				
21				
22				
22				
23				
23				
24				
24				
25	32, 29, 34	3.2×10^7	3.3×10^7	7.5185
25	41, 31, 27	3.3×10^7		
26	33, 28, 51	3.7×10^7	4.5×10^7	7.6532
26	56, 54, 46	5.2×10^7		
27	43, 42, 53	4.6×10^7	4.3×10^7	7.6335
27	36, 47, 36	4.0×10^7		
28	52, 45, 45	4.7×10^7	3.9×10^7	7.5911
28	28, 32, 32	3.1×10^7		
41				
41				
43				
43				
44				
44				
47				
47				
48				
48				
49				
49				

(continued)

TABLE 57

Irradiated chicken meat

Dose: 3×10^6 rads of ionizing radiations

(continued)

Incubation time (hrs.	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 3		
21	208,186,214	2.03×10^7		
21	36, 32, 32	3.4×10^7	2.72×10^7	7.4346
22	43, 41, 26	3.7×10^7		
22	126,133,127	1.29×10^7	2.50×10^7	7.3979
23	46, 33, 37	3.9×10^7		
23	33, 34, 36	3.4×10^7	3.7×10^7	7.5682
24				
24				
25				
25				
26				
26				
27				
27				
28				
28				
41	45, 39, 44	4.3×10^7		
41	54, 56, 52	5.4×10^7	4.9×10^7	7.6902
43	54, 66, 60	6.0×10^7		
43	78, 64, 70	7.1×10^7	6.6×10^7	7.8195
44	55, 68, 54	5.9×10^7		
44	107,105, 98	1.03×10^8	8.1×10^7	7.9085
47				
47				
48				
48				
49				
49				

TABLE 58

Irradiated chicken meat

Dose: 6×10^6 rads of ionizing radiations*

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 1		
24	62, 56, 59	5.9×10^7		
24	33, 44, 45	4.1×10^7	5.0×10^7	7.6990
26	226,235,223	2.28×10^7		
26	105, 80,109	9.8×10^7	6.0×10^7	7.7782
27	49, 61, 61	5.7×10^7		
27	30, 39, 30	3.3×10^7	4.5×10^7	7.6532
28	57, 54, 66	5.9×10^7		
28	72, 48, 70	6.3×10^7	6.1×10^7	7.7853
29	35, 54, 53	4.7×10^7		
29	58, 37, 43	4.6×10^7	4.7×10^7	7.6721
38				
38				
40				
40				
45				
45				
47				
47				
49				
49				
		SET 2		
24				
24				
26				
26				
27	30, 35, 45	3.7×10^7		
27	47, 37, 42	4.2×10^7	4.0×10^7	7.6021
28	41, 31, 34	3.5×10^7		
28	71, 79, 71	7.4×10^7	5.5×10^7	7.7404
29	82, 80, 82	8.1×10^7		
29	54, 66, 50	5.7×10^7	6.9×10^7	7.8388
38				
38				
40				
40				
45				
45				
47				
47				
49				
49				

(continued)

*Ionizing radiations produced by a 2 Mev Van de Graaff accelerator.

TABLE 58

Irradiated chicken meat

Dose: 6×10^6 rads of ionizing radiations

Incubation time (hrs.)	BHI agar plate count	Mean of three plate counts org/disc	Mean of two discs org/disc	Log_{10} mean
		SET 3		
24				
24				
26				
26				
27				
27				
28				
28				
29				
29				
38	276,264,258	2.66×10^8	1.83×10^8	8.2625
38	98,113, 86	9.9×10^7		
40	203,215,221	2.13×10^8	1.40×10^8	8.1461
40	71, 67, 63	6.7×10^7		
45	68, 70, 81	7.3×10^7	1.28×10^8	8.1072
45	195,177,175	1.82×10^8		
47	301,312,296	3.03×10^8	2.15×10^8	8.3324
47	127,125,127	1.26×10^8		
49	132,127,124	1.29×10^8	1.50×10^8	8.1761
49	166,174,172	1.71×10^8		

APPENDIX II

STATISTICAL PROCEDURES

A. Symbols Used

x = time in hours

y = number of organisms or $\log_{10}$ number of organisms

n = number of samples

S = sum of observations

SS = sum of squares of observations

SSD = sum of squares of differences

f = degrees of freedom

s^2 = variance

b = slope of line

SP = sum of products

SPD = sum of product differences

$y|x$ = values of y when x is constant

S^2_p = pooled variance

$\bar{y}$ = mean of more than one observation of y

$\bar{x}$ = mean of more than one observation of x

k = number of groups

N = sum of n values

s = standard deviation

t = value extracted from Student's tables of t

F = value extracted from tables of F

Regression analysis computing table

	Observations	n	S	SS	s^2/n	SSD	f	s^2	nx	x^2	nx^2
x_1	$y_{11}, y_{12}, \ldots, y_1n_1$	n_1	S_1	SS_1	S_1^2/n_1	SSD_1	n_1-1	$s^2_{1_1}$	n_1x_1	x_1^2	$n_1x_1^2$
x_2	$y_{21}, y_{22}, \ldots, y_2n_2$	n_2	S_2	SS_2	S_1^2/n_2	SSD_2	n_2-1	$s^2_{2_1}$	n_2x_2	x_2^2	$n_2x_2^2$
.	. . . .	.	.	.	.	.	.	.	.	.	.
.	. . . .	.	.	.	.	.	.	.	.	.	.
.	. . . .	.	.	.	.	.	.	.	.	.	.
x_k	$y_{k1}, y^k_2, \ldots, y_kn_k$	n_k	S_k	SS_k	S_k^2/n_k	SSD_k	n_k-1	$s^2_{k_1}$	n_kx_k	x_k^2	$n_kx_k^2$
TOTAL		$\sum n_i$	$\sum S_i$	$\sum SS_i$	$\sum S_i^2/n_i$	$\sum SSD_i$	$\sum n_i-k$		$\sum n_ix_i$		$\sum n_kx_k^2$

1. $N = \sum n_i$
2. $S_x = \sum n_i x_i$
3. $SS_x = \sum n_i x_i^2$
4. S_x^2/N
5. $SSD_x = SS_x - S_x^2/N$
6. $S\bar{y} = \sum n_i\bar{y}_i = \sum S_i$
7. $SS\bar{y} = \sum n_i\bar{y}_i^2 = \sum S_i^2/n_i$
8. $S\bar{y}^2/N$
9. $SSD\bar{y} = SS\bar{y} - S\bar{y}^2/N$
10. $SP_{x\bar{y}} = \sum n_ix_i\bar{y}_i = \sum x_iS_i$
11. $S_xS_{\bar{y}}/N$
12. $SPD_{x\bar{y}} = SP_{x\bar{y}} - S_xS_{\bar{y}}/N = \sum n_i(x_i-\bar{x})(\bar{y}_i-\bar{y})$
13. $b = SPD_{x\bar{y}}/SSD_x$
14. $SPD^2_{x\bar{y}}/SSD_x = bSPD_{x\bar{y}}$
15. $SSD_{\bar{y}|x} = SSD_{\bar{y}} - SPD^2_{x\bar{y}}/SSD_x = \sum n_i(\bar{y}_i-Y_i)^2, f = k-2$
16. $SSD_{y|x} = \sum SSD_i + SSD_{\bar{y}|x}, \quad f = N-2$
17. $s^2 = SSD_{\bar{y}|x}/N-2$

B. Procedure for regression analysis

Regression analysis was utilized to determine the best straight line for the growth response of microorganisms during the logarithmic phase of growth. The analysis was performed by preparation of the regression analysis computing table (on the preceding page) and subsequent determination of the required values listed below the table.

When two or more slopes were obtained from repeated experiments in which the variables were the same, it was assumed that the results were statistically the same and the slope values were combined to form an average slope value by use of the following formula:

18. $$\overline{b} = \frac{SPD_{x_1y_1} + SPD_{x_2y_2} + \cdots\cdots + SPD_{x_ny_n}}{SSD_{x_1} + SSD_{x_2} + \cdots\cdots SSD_{x_n}}$$

Combined terms were then formed for use in future slope comparisons with samples which had been subjected to different treatments. The following formulae illustrate how the combined terms were obtained.

19. $$\overline{SSD}_x = SSD_{x_1} + SSD_{x_2} + \cdots\cdots SSD_{x_n}$$

20. $$\overline{N} = N_1 + N_2 + \cdots\cdots + N_n$$

21. $$\overline{s}^2 = \frac{(N_1-2)s_1^2 + (N_2-2)s_2^2 + \cdots\cdots + (N_n-2)s_n^2}{N_1 + N_2 + \cdots\cdots N_n - 2n}$$

The standard error and the 95% confidence intervals of the average slope were determined by use of the following formulae:

22. $$S.E. = \sqrt{\frac{\bar{s}^2}{\overline{SSD}_x}}$$

23. 95% confidence interval = S.E. x $t_{.05}$
t having $\bar{N} - 2n$ degrees of freedom.

C. Procedure for the comparison of two slopes

After the slopes of the logarithmic growth phase curves were determined, comparisons were made to determine the significance of the differences observed between samples subjected to various treatments.

The comparisons were made by preparation of the following slope comparison computing table and subsequently performing an F test of the variance ratio followed by a t test of the two slopes, provided that the variance ratio was insignificant. If the slopes were not found to be significantly different, they could be pooled to form a mean slope. The formulae employed in these tests are presented below the computing table.

Slope comparison computing table

$\bar{N}_1$	$\bar{b}_1$	$\overline{SSD}_{x_1}$	$f_1=\bar{N}_1-2_{n_1}$	$\bar{s}_1^2$
$\bar{N}_2$	$\bar{b}_2$	$\overline{SSD}_{x_2}$	$f_2=\bar{N}_2-2_{n_2}$	$\bar{s}_2^2$

Variance ratio:

$$24.\quad F = \frac{\bar{s}^2_{larger}}{\bar{s}^2_{smaller}} \qquad F_{.99}(f_{larger},\ f_{smaller}) =$$

Pooled variance:

$$25.\quad s_p^2 = \frac{(\bar{N}_1-2n)_1\bar{s}_1^2 + (\bar{N}_2-2n)_2\bar{s}_2^2}{\bar{N}_1+\bar{N}_2 - 2(n_1+n_2)}$$

t test:

$$26.\quad t = \frac{\bar{b}_1 - \bar{b}_2}{s_p\ \sqrt{1/\overline{SSD}_{x_1} + 1/\overline{SSD}_{x_2}}}$$

D. Procedure to test the hypothesis that several populations have equal variance

Many statistical procedures are based upon the assumption of equal variance between samples or groups of samples being compared. A test was developed by Bartlett (1937), modified by Box (1949), and presented by Dixon and Massey (1951). The source of values for the performance of the test may be either a regression analysis computing table or a variance analysis computing table. The test is carried out as follows:

$$27.\quad \text{Let } M = (N-k)\ln s_p^2 - \sum\left[(n_i-1)\ln s_i^2\right]$$

$$28.\quad s_p^2 = \frac{\sum(n_i-1)s_i^2}{N-k} = \frac{SSD}{N-k}$$

29. $A = \frac{1}{3(k-1)} \left[\sum'(\frac{1}{n_i-1}) - \frac{1}{N-k}\right]$

30. $f_1 = k-1$

31. $f_2 = \frac{k+1}{A^2}$

32. $d = \frac{f_2}{1-A+(2/f_2)}$

Then the sampling distribution of $F = \frac{f_2 M}{f_1(d-M)}$ is approximately $F(f_1,f_2)$.

E. Procedure for analysis of variance with a single variable of classification

Groups of samples which have been subjected to a single variation in treatment may be subjected to analysis of variance tests to determine the significance of the differences between means observed for different treatments. The evaluation was performed by preparation of the following analysis of variance computing table from which values could be obtained for the formulae listed below the table. The analysis of variance table was then prepared and the results of the analyses were obtained through the use of the formulae which appear below that table.

Analysis of variance computing table

Observations	n	S	SS	S^2/n	SSD	f	s^2
$y_{11}, y_{12}, \ldots y_{1n_1}$	n_1	S_1	SS_1	S_1^2/n_1	SSD_1	n_1-1	s_{11}^2
$y_{21}, y_{22}, \ldots y_{2n_2}$	n_2	S_2	SS_2	S_2^2/n_2	SSD_2	n_2-1	s_{21}^2
. . .	.	.	.	.	.	.	.
. . .	.	.	.	.	.	.	.
. . .	.	.	.	.	.	.	.
$y_{k_1}, y_{k_2}, \ldots y_{kn_k}$	n_k	S_k	SS_k	S_k^2/n_k	SSD_k	n_k-1	$s^2{}_{k_1}$
TOTAL	Σn_i	ΣS_i	ΣSS_i	$\Sigma S_i^2/n_i$	ΣSSD_i	Σn_i-k	Σs_i^2

33. $Sy = \Sigma S_i$

34. $SSy = \Sigma SS_i$

35. $S_{\bar{y}}^2/N = \dfrac{(\Sigma S_i)^2}{N}$

36. $SSD_{\bar{y}} = SS_y - S_y^2/N$

37. $\Sigma SSD = \Sigma[SS_i - S_i^2/n]$

38. $\Sigma SS_i - S_{\bar{y}}^2/N = \Sigma SS_i - \dfrac{(\Sigma S_i)^2}{N}$

Analysis of variance table

	Sum of squares	Degrees of freedom	Mean square
means	$SSD_{\bar{y}}$	k-1	$SSD_{\bar{y}}/k-1$
within	$\sum SSD$	N-k	$\sum SSD/N-k$
Total	$\sum SS_i - S_{\bar{y}}^2/N$		

Test for F ratio:

$$F = \frac{\text{mean square for means}}{\text{mean square for within}} =$$

Critical F ratio:

$$F_{.95}(k-1, N-k) =$$

F. Procedure for analysis of variance with two variables of classification and repeated measurements

Groups of samples which have been subjected to dual treatment variation may be compared with one another in order to determine the significance of differences in observed means. In this case, an analysis of variance test was used. The values for the analysis of variance tables were obtained from an analysis of variance computing table which was previously presented. The following values were required for the preparation of the analysis of variance tables:

39. $SS_t = SS_1 + SS_2$

40. $S_t = S_1 + S_2$

41. $S_t^2/N_t = (S_1 + S_2)^2/N_1 + N_2$

42. $SSD_t = SS_t - S_t^2/N_t$

43. $$\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2} - \frac{S_t^2}{N_1 + N_2}$$

44. $$\frac{\sum(S_{i_1} + S_{i_2})^2}{\sum(n_{i_1} + n_{i_2})} - \frac{S_t^2}{N_1 + N_2}$$

45. $$\left[\sum \frac{(S_{i_1})^2}{n_{i_1}} + \sum \frac{(S_{i_2})^2}{n_{i_2}}\right] - \frac{S_t^2}{N_1 + N_2}$$

Analysis of variance table; interaction

	Sum of squares	Degrees of freedom	Mean square
Row means	$\frac{S_1^2 + S_2^2}{N_1 + N_2} - \frac{S_t^2}{N_t}$	No. of rows - 1	$\frac{\text{sum of squares}}{\text{degrees of freedom}}$
Column means	$\left(\frac{S_{i_1} + S_{i_2}}{n_{i_1} + n_{i_2}}\right)^2 - \frac{S_t^2}{N_1 + N_2}$	No. of columns - 1	$\frac{\text{sum of squares}}{\text{degrees of freedom}}$
Interaction	Subtotal-(row mean+ column means)	Difference	$\frac{\text{sum of squares}}{\text{degrees of freedom}}$
Subtotal	$\sum \frac{(S_{i_1})^2}{n_{i_1}} + \sum \frac{(S_{i_2})^2}{n_{i_2}} - \frac{S_t^2}{N_1 + N_2}$	No. of subtotals - 1	-----
Within groups	SSD_t - subtotal	Difference	$\frac{\text{sum of squares}}{\text{degrees of freedom}}$
Total	SSD_t	$N_t - 2$	-----

Test for interaction:

$$F = \frac{\text{mean square interaction}}{\text{mean square within groups}} = \quad \bullet \quad F_{.95}(f_{\text{int.}}, f_{\text{within}}) =$$

If the interaction term proved insignificant, the test for equality of means was performed by preparation of another analysis of variance table in which the interaction term is combined with the within groups term to form a residual term.

Analysis of variance table

	Sum of squares	Degrees of freedom	Mean square
Rows	$\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2} - \frac{S_t^2}{N_t}$	No. of rows - 1	$\frac{\text{sum of squares}}{\text{degrees of freedom}}$
Columns	$(\frac{S_{i_1}+S_{i_2}}{n_{i_1}+n_{i_2}})^2 - \frac{S_t^2}{N_1+N_2}$	No. of columns - 1	$\frac{\text{sum of squares}}{\text{degrees of freedom}}$
Residual	Total - (rows + columns)	Difference	$\frac{\text{sum of squares}}{\text{degrees of freedom}}$
Total	SSD_t	N_t-2	

Test for row effects:

$$F = \frac{\text{mean square rows}}{\text{mean square residual}}, \qquad F_{.95}(f_R, f_{Re}) =$$

Test for column effects:

$$F = \frac{\text{mean square columns}}{\text{mean square residual}}, \qquad F_{.95}(f_C, f_{Re}) =$$

G. Sample calculations

1. Regression analysis

The data used in the sample presented below is Set 1 of the tables for the logarithmic growth of Escherichia coli on raw chicken meat at 37°C which appeared in Appendix I, Section C.

The slope of the best straight line was determined.

SET 1

Regression analysis computing table

x	Observations y_1	y_2	y_3	n	S	SS	S^2/n	SSD	f	s^2	nx	x^2	nx^2
4	5.2041	5.4249	5.2480	3	15.8770	84.053701	84.026376	.027325	2	.013663	12	16	48
5	5.3560	5.2967	5.5051	3	16.1578	87.047893	87.024834	.023059	2	.011530	15	25	75
6	6.2455	6.2014	6.3579	3	18.8048	117.886525	117.873501	.013024	2	.006512	18	36	108
7	6.7482	6.6232	6.8325	3	20.2039	136.088038	136.065858	.022180	2	.011090	21	49	147
8	7.0899	7.5441	7.3560	3	21.9900	161.290863	161.186700	.104163	2	.052082	24	64	192
	TOTALS			15	93.0335		586.177269	.189751	10		90		570

1. $N = 15$
2. $S_x = 90$
3. $SS_x = 570$
4. $S_x^2/N = 540$
5. $SSD_x = 30$
6. $S_{\bar{y}} = 93.0335$
7. $SS_{\bar{y}} = 586.177269$
8. $S_{\bar{y}}^2/N = 577.015475$
9. $SSD_{\bar{y}} = 9.161794$
10. $SP_{x\bar{y}} = 574.47310$
11. $S_x S_{\bar{y}}/N = 558.20100$
12. $SPD_{x\bar{y}} = 16.2721$
13. $b = 0.5424$
14. $SPD_{x\bar{y}}/SSD_x = 8.826041$
15. $SSD_{\bar{y}|x} = 0.335753$
16. $SSD_{y|x} = 0.525505$
17. $s^2 = .04042$
    $f = 13$

2. Average of two slopes

The data used in the sample calculations presented below were presented in Appendix I, Section C, and describe the logarithmic growth of Escherichia coli on raw chicken meat at 37°C.

The computing table for Set 1 was presented previously in the regression analysis section of sample calculations. The computing table for Set 2 is presented below. Sub 1 refers to data from Set 1. Sub 2 refers to data from Set 2.

The average slope and the 95% confidence interval for this average slope were determined.

SET 2

Regression analysis computing table

x	Observations y_1	y_2	y_3	n	S	SS	S^2/N	SSD	f	s^2	nx	x^2	nx^2
4	5.0000	4.4771	4.7076	3	14.1847	67.205922	67.068571	.137351	2	.068676	12	16	48
5	4.9685	5.4200	4.9542	3	15.3427	78.606490	78.466148	.140342	2	.070171	15	25	75
6	5.5798	6.1959	5.8692	3	17.6449	103.970854	103.780832	.190022	2	.095011	18	36	108
7	6.3692	6.2355	5.9138	3	18.5185	114.421199	114.311614	.109585	2	.054793	21	49	147
8	6.9294	7.0043	6.7243	3	20.6580	142.293013	142.250988	.042025	2	.021013	24	64	192
	TOTALS			15	86.3488		505.878153	.619325	10		90		570

1. $N = 15$
2. $S_x = 90$
3. $SS_x = 570$
4. $S_x^2/N = 540$
5. $SSD_x = 30$
6. $S_{\bar{y}} = 86.3488$
7. $SS_{\bar{y}} = 505.878153$
8. $S_{\bar{y}}^2/N = 497.074350$
9. $SSD_{\bar{y}} = 8.803803$
10. $SP_{x\bar{y}} = 534.2152$
11. $S_x S_{\bar{y}} = 518.0928$
12. $SPD_{x\bar{y}} = 16.1224$
13. $b = 0.53741$
14. $SPD^2_{x\bar{y}}/SSD_x = 8.664393$
15. $SSD_{\bar{y}|x} = 0.139410$
16. $SSD_{y|x} = 0.758735$
17. $s^2 = 0.05836$

18. $\overline{b} = \frac{16.2721 + 16.1224}{30 + 30} = 0.5399$

19. $\overline{SSD}_x = 30 + 30 = 60$

20. $\overline{N} = 15 + 15 = 30$

21. $\overline{s}^2 = \frac{(15-2)(.04042)+(15-2)(.05836)}{15 + 15 - 4} = 0.04939$

Standard error and 95% confidence interval of the average slope:

22. $S.E. = \sqrt{\frac{.04939}{60}} = 0.02869$

23. 95% confidence interval with 26 degrees of freedom:

$0.02869 \times \pm 2.06 = \pm 0.0591$

3. Comparison of two average slopes

The data used in the sample calculations below were presented in Appendix I, Section C, and describe the logarithmic growth of E. coli on raw chicken meat and on heat processed chicken meat at 37°C. The computing tables for the regression analyses of the raw chicken data were presented previously in Appendix II, Sections G1 and G2. The computing tables for the regression analyses of the heat processed chicken will not be presented, but the values required for the forthcoming calculations will be presented in the following slope comparison computing table. Sub 1 refers to data from raw chicken samples. Sub 2 refers to data from heat processed chicken.

Slope comparison computing table

	$\overline{N}$	$\overline{b}$	$\overline{SSD_x}$	f	$\overline{s^2}$
sub 1	30	0.5399	60	26	.04939
sub 2	22	0.6883	69.4	18	.05152

24. $F = \frac{0.05152}{0.04939} = 1.043$ $\qquad F_{.99}(18, 26) = 2.70$

variances are homogeneous

25. $s_p^2 = \frac{(30-4)(.04939)+(22-4)(.05152)}{30 + 22 - 2(2+2)} = 0.05026$

26. $t = \frac{.6883 - .5399}{.22419 \sqrt{1/60 + 1/69.4}} = 3.76$

$t_{.05}(44) \cong 2.02$

$t_{.005}(44) \cong 2.97$

differences are highly significant

4. Equal variance by the method of Box (1949)

The data used in the sample calculation presented below are the results obtained from dilution of drops of inoculum in a series of 99 milliliter dilution blanks. The data originally appeared in Table 6 of the text.

Direct Dilution

Analysis of variance computing table

Observations												
y_1	y_2	y_3	y_4	y_5	y_6	y_7	n	S	SS	S^2/n	SSD	f	s^2
68	67	58					3	193	12477	12416.33	60.67	2	30.335
67	77	76					3	220	16194	16133.33	60.67	2	30.335
73	68	63					3	204	13922	13872.00	50.00	2	25.00
83	75	80	58	68	79	60	7	503	36743	36144.14	598.86	6	99.667
79	70	70	62	56	59	64	7	460	30598	30228.57	369.43	6	61.572
			TOTALS				23	1580	109934	108794.37	1139.63	18	246.909

Hypothesis variances are equal

27. $s_p^2 = \frac{1139.63}{18} = 63.3127$

28. $M = 18(4.14789) - [2 \ln 30.335 + 2 \ln 30.335 + 2 \ln 25 + 6 \ln 99.667 + 6 \ln 61.572]$

$M = 18(4.14789) - [2(3.41115) + 2(3.41115) + 2(3.21888) + 6(4.60176) + 6(4.12017)]$

$M = 74.66202 - [6.82230 + 6.82230 + 6.43776 + 27.61056 + 24.72102]$

$M = 74.66202 - 72.41394 = 2.24808$

29. $A = \frac{1}{3(4)}\left[\frac{1}{2} + \frac{1}{2} + \frac{1}{2} + \frac{1}{6} + \frac{1}{6} + \frac{1}{18}\right]$

30. $A = \frac{1}{12}\left[\frac{11}{6} - \frac{1}{18}\right] = \frac{1}{12}\left[\frac{32}{18}\right] = \frac{32}{216} = 0.14814$

30. $f_1 = 5 - 1 = 4$

31. $f_2 = 5 + 1/A^2 = 6/.02195 = 273.348$

32. $d = \frac{273.348}{1 - .14814 + .00732} = \frac{273.348}{0.85918} = 318.1498$

$F = \frac{(273.348)(2.24808)}{4(318.1498 - 2.24808)} = \frac{614.50817}{1263.60688} =$

0.486

$F^{95}_{(4,280)} = 2.37$

5. Analysis of variance: single variable of classification

The data used in the sample calculation presented below are the results obtained from the dilution of drops of inoculum in a series of 99 milliliter dilution blanks. The data originally appeared in Table 6 of the thesis.

The computing table is the same one which was presented in the previous section 3.

Analysis of variance table

	Sum of squares	d.f	mean square	F ratio test
means	255.24	4	63.81	$F=63.81/63.31$ $F = 1.008$
within	1139.63	18	63.31	$F_{.95}(4,18)=2.93$
total	1394.87	22		

33. $S_y = 1580$

34. $SS_y = 108794.37$

35. $s_{\overline{y}}^2/N = 108539.13$

36. $SSD_{\overline{y}} = 255.24$

37. $\Sigma SSD = \Sigma SS_i - \Sigma S_i^2/n = 1139.63$

38. $\Sigma SS_i - S_{\overline{y}}^2/N = 109934 - 108539.13 = 1394.87$

6. Analysis of variance: two variables of classification, repeated measurements

The data used in the sample calculation presented below were presented in Table 6 of the thesis. The experiment compared the number of organisms in a series of drops, some of which were diluted directly in 99 milliliter dilution blanks, others of which were first spread across the surface of solid substrate discs and subsequently diluted in 99 milliliter dilution blanks by means of a grinding operation.

Some of the values required in the following calculations were presented in the computing table of the previous section, 3. The remainder are presented in the computing table below.

Sub 1 values represent data from drops which were directly inoculated into dilution blanks. Sub 2 values represent data from drops which were first spread over the surface of a solid substrate and then suspended in dilution blanks.

Solid Substrate

Analysis of variance computing table

y_1	y_2	y_3	y_4	y_5	y_6	n	S	SS	S^2/n	SSD	f	s^2
54	66	72				3	192	12456	12288	168	2	84.0
63	51	73				3	187	11899	11656.33	242.67	2	121.335
59	71	60				3	190	12122	12033.33	88.67	2	44.335
62	81	72	76	93	60	6	444	33614	32856	758	5	151.60
79	71	83	71	76	65	6	445	33213	33004.17	208.83	5	41.766
		TOTALS				21	1458	103304	101837.83	466.17	16	433.036

39. $SS_t = 109934 + 103304 = 213238$

40. $S_t = 1580 + 1458 = 3038$

41. $S_t^2/N_t = S_t^2/44 = 209760$

42. $SSD_t = 213238 - 209760 = 3478$

43. $S_1^2/N_1 + S_2^2/N_2 - S_t^2/N_t = \frac{(1580)^2}{23} + \frac{(1458)^2}{21} - 209760 = 5.987$

44. $\sum(\frac{S_{i_1}+S_{i_2}}{n_{i_1}+n_{i_2}})^2 - \frac{S_t^2}{N_1+N_2} = \left[\frac{(193+192)^2}{6} + \frac{(220+187)^2}{6} + \frac{(204+190)^2}{6} + \frac{(503+444)^2}{13} + \frac{(460+445)^2}{13} - 209760\right] = 412.232$

45. $\left[\sum\frac{(S_{i_1})^2}{n_{i_1}} + \sum\frac{S_{i_2}^2}{n_{i_2}}\right] - \frac{S_t^2}{N_1+N_2} = \left[108794.37 + 101837.83\right] - 209760 = 872.2$

Analysis of variance table: interaction

	Sum of squares	df	Mean square
Row means	5.987	1	5.987
Column means	412.232	4	103.058
Interaction	453.981	4	103.495
Subtotal	872.2	9	96.9
Within groups	2605.8	34	76.6412
Total	3478.0	43	

Test for significant interaction:

$$F = \frac{103.495}{76.6412} = 1.3504 \qquad F_{95}(4, 34) = 2.61 - 2.69$$

no significant interaction

Analysis of variance table

	Sum of squares	df	Mean square
Rows	5.987	1	5.987
Columns	412.232	4	103.058
Residual	3059.781	38	80.5206
Total	3478.000	43	

Test for row effects:

$$F = \frac{5.987}{80.5206} = .0744 \qquad F_{95}(1, 38) = 4.08 - 4.17$$

no row effects

Test for column effects:

$$F = \frac{103.058}{80.5206} = 1.2799 \qquad F_{95}(4, 38) = 2.61 - 2.69$$

no column effects

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BIOGRAPHICAL SKETCH

Myron Solberg was born in Boston, Massachusetts on June 11, 1931. He was educated in the Boston public school system and was graduated from the Boston Latin School in 1948. He entered the University of Massachusetts where he majored in Food Technology, and earned the degree of Bachelor of Science in 1952. In August, 1952 he was placed on extended active duty as a Second Lieutenant in the United States Air Force. He served as a Supply Officer, Material Facilities Officer, and Management and Procedures Officer during a two year tour of duty in the United States and in Newfoundland, Canada. He is currently a Research and Development Officer, Special in the Air Force Reserve with the permanent rank of Captain.

After honorable separation from the United States Air Force he entered the Department of Food Technology at the Massachusetts Institute of Technology as a doctoral candidate. As a part-time Division of Industrial Cooperation employee (September 1954-March 1956), he carried out research directed toward the study of the comparative radiobiological effects of intermittent vs. continuous beams of ionizing radiations. As a part-time employee of the Department of Food Technology (March 1956-September 1958), he carried out investigations relating to the comparison of Vitamin C determination in soups by chemical titration and by guinea pig assay methods, and analyses of the amino acid content of Maine sardines which were subjected to the various processing procedures employed by the industry. He was appointed a Research Assistant in the Department of Food Technol-

ogy in September 1958 and was assigned to the Microbiology Research Laboratory to conduct research pertaining to radiation effects upon organisms of public health significance, under grant E-987 from the National Institutes of Health.

The author is a member of Sigma Xi, the Institute of Food Technologists, and the American Association for the Advancement of Science.

He is co-author of the following scientific publication: Myron Solberg and B.E. Proctor, A technique utilizing 2,3,5 triphenyltetrazolium chloride for recognition of bacterial colonies in the presence of large numbers of food particles. Food Technology, 1960 (in press - scheduled for publication in July, 1960).