A BACTERIOLOGICAL SURVEY OF RAPID CURED PRE-RIGOR HAM

BY

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Bachelor of Science

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Morgantown, West Virginia

1957

Submitted to the faculty of the Graduate School
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1966

A BACTERIOLOGICAL SURVEY OF RAPID CURED PRE-RIGOR HAM

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ACKNOWLEDGMENT

The author wishes to express appreciation to Dr. R. L. Henrickson, Department of Animal Science, for continued guidance, assistance and encouragement in this research.

Grateful acknowledgment is also extended to Dr. E. C. Noller, of the Department of Microbiology, for assistance and counsel throughout the research preparation of this thesis.

The author wishes to acknowledge the following persons for their willingness to discuss specific problems in connection with the preparation of this manuscript: Dr. L. E. Walters, Department of Animal Science; Dr. H. C. Olsen, Department of Dairy Science; and Drs. P. B. Barto and R. E. Corstvet, Department of Veterinary Parasitology and Public Health. Recognition is accorded Mrs. M. McCants for technical assistance and to Mr. R. W. Mandigo for assistance with the statistical analyses and various phases of applied chemical methodology.

Special appreciation is given to my wife, Marlene, and children, Kathy Sue, and Charles, Jr., for their assistance and faithful encouragement throughout this program of graduate study.

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INTRODUCTION

Through the ages, cured ham has traditionally enjoyed a prolonged period of stability over various forms of fresh meat. This has been attributed to the complex biochemical and physicochemical relationship which exist inherently within the cured tissue and results in extended stability, modified color, and enhanced organoleptic characteristics. The complexity of this relationship has been widely exploited and subjected to innumerable investigations.

Present industry practice relies on extensive post-slaughter chilling to retard bacterial growth and maintain quality in ham curing operations. Current trends toward increased residual moisture and lowered salt content in ready-to-eat ham causes one to question the limit and the desirability of this practice. Nevertheless, with the evolutionary advancements made in curing and processing techniques coupled with more rigid sanitary control and widespread availability of refrigeration, the modern light-cured, boneless, smoked, and ready-to-eat ham has been widely acclaimed and accepted by the convenience-minded consumer.

Processing of ham to a finished form prior to initial chilling has introduced a radically new concept to the meat industry. Several investigations have shown the desirability of rapid, hot-processing techniques in ham curing operations. However, since these techniques eliminate conventional chilling, bacteriological conditions of the product are questioned. Therefore, the objectives of the research undertaken were to: (1) quantitatively compare the bacterial load common to conventional and

hot processed ready-to-eat ham and (2) to study the incidence qualitatively in order that predictions relative to possible quality detriment, shelf life or potential hazard(s) to public health could be made.

The ham was selected as the source for bacteriological samples because it offers a large, thick muscle mass and is representative of a wholesale cut receiving the majority of post-slaughter processing and curing manipulation. Since it has been well established that considerable variation exists among the various ham muscles regarding physiological and histological conditions at any constant point in time, no attempt was made to secure homogenous samples from a single muscle. However, every attempt was made to secure a representative portion of the deep tissues from the center ham region.

REVIEW OF LITERATURE

Information reported herein will be confined, in general, (1) to those intrinsic and extrinsic factors responsible for the selective bacteriostatic action within the cured meat tissue, and (2) to the status of knowledge relative to explaining the existance of an associative bacterial flora in present day smoked and fully cooked cured ham. The review will not be confined to porcine meat tissue in explaining or eliciting the facts from numerous other investigations, but will include those points considered relevant involving other processed foods from other species in an attempt to draw on all the existing knowledge.

Development of the Modern Ready-to-Eat Ham

According to Bratzler (1958), Wilson and Company in 1937 is credited with developing the first mild flavored ham which was cured rapidly via artery pumping and heat processed so that it could be eaten after only limited heating. A product labeled "ready-to-eat" under United States Department of Agriculture (U.S.D.A.) inspection must be maintained at an internal temperature of not less than 140°F for a minimum of 30 minutes and produce a smoked flavor not induced by a chemical or smoke substitute (Levie, 1963). The present day buffet type ham is characterized by Levie (1963) as being completely boned, rolled and formed back into what is roughly a ham shape by a fibrous casing. This type of ham must have a statement such as "keep refrigerated" prominently displayed on the label in order to meet

U.S.D.A. regulations governing meat inspection requirements (U.S.D.A., 1959). The perishable nature of this modern "cured" product has evolved as a result of various modifications in ham curing and processing techniques. Authorization for incorporating polyphosphates into curing brines in 1952 facilitated the production of a juicier, more desirable ham (Brissey, 1952). Curing processes were further advanced in 1954 and 1955 by the approval of ascorbic acid and sodium ascorbate, respectively, as rapid cure adjuncts (Miller, 1954, 1955). These developments coupled with the widespread availability of refrigeration enabled the industry to reduce curing time from several weeks to a matter of hours.

Mullins et al. (1957) investigated the effect of simultaneous injection of an ascorbate, hexametaphosphate, and calcium lactate-lactic acid mixture on cure penetration, color development and stability, and palatability factors in hams which were treated before and after chilling. These workers reported that hams injected hot were slightly more uniform in color than paired hams injected after being chilled. In addition, no appreciable difference in palatability factors were noted in the treated hams. More recently, Weiner (1964) studied the effect of processing pork carcasses prior to the completion of rigor mortis on certain quality characteristics of muscle and fat. Mandigo and Henrickson (1965a, 1965b) demonstrated the feasibility of processing both hams and bacons by rapid, hot processing techniques. Parker (1952) has described conventional handling of "ready-to-eat" type ham in terms of the smoking and cooking requirements and methods for its proper cooling and handling after being processed. The similarity of these methods to the present practices being followed by the industry is remarkable and points out the longstanding dependence

upon extensive refrigeration capability and maintenance of correct temperature(s) and humidity throughout the processing schedule. Heck (1955) reported that delay in chilling pork carcasses resulted in lower quality cured meat and appeared to increase the chance for incipient spoilage.

Due to the increased emphasis on organoleptic characteristics and the trend toward closer quality control, a number of bacteriological investigations were made to determine the role of certain bacteria in ham curing operations (Sulzbacher, 1957; Deibel and Niven, 1958; and McLean and Sulzbacher, 1959). Deibel and Niven (1958), after a detailed study of ham curing bacteria, reported that none of their isolates bore any significance to the curing process. McLean and Sulzbacher (1959), however, advocated the importance of certain species of bacteria in curing brines because of the production of specific flavor and aroma characteristics in hams studied under their controlled experimental conditions. It was formerly thought that certain bacteria along with fermentable carbohydrate optimized reducing conditions for proper cure color development; however, with the utilization of chemical reductants much of the uncertainty formerly associated with curing operations has been eliminated (Niven, 1960). Rose and Peterson (1953) showed that the effects of pH and temperature on nitrite destruction did not parallel the effect on bacterial growth, and that bacterial numbers became negligible below pH 5.0 or above 113°F (45°C). Niven (1960) has noted that the industry trend is to eliminate the necessity for bacterial reduction of nitrate in curing operations. Shank et al. (1962) suggested that the formation of nitrous acid, via a pH-dependent mechanism, was responsible for the quantitative as well as qualitative changes which

occurred in the bacterial flora of cured meat. Consequently, today's processing objective is to eliminate all bacteria from the product and preclude their development from within by selective bactericidal activity and close temperature control. Batcher et al. (1964) implied that today's commercially processed quick-cured hams are of better eating quality because they contain more moisture and a lower salt content.

Factors Affecting the Bacterial Load in Uncured Ham

The American Meat Institute Foundation (A.M.I.F.) has established that after slaughter, animal tissues continue to support changes of glycogen to lactic acid, fats become oxidized and hydrolyzed and various enzymes commence their autolytic activity thus altering the biochemical, physicochemical, and structural nature of the substrate (A.M.I.F., 1960). The degree of these changes and the rate at which they occur undoubtedly influences the bacteriology of the fresh tissue.

As a result of death, the natural defense mechanisms possessed by the normal healthy animal are destroyed. It has been shown that bleeding may initially introduce bacteria into the circulatory system of the immobolized animal (Jensen and Hess, 1941). Early workers (McBryde, 1911; Reith, 1926 and Jensen and Hess, 1941), clearly established the presence of certain bacteria in fresh ham tissue. Ayres (1955) expresses the following opinion relative to the bacteriology of the meat animal:

> Whether or not the load of microorganisms associated with the living animal contributes a significant share of the contamination occurring in and on the carcass depends not only upon the methods of handling that the meat receives but is determined also by the interrelation between the defensive mechanisms of the animal and the enormous microbial populations which gain access to the animal.

From the work of Lepovetsky et al. (1953) it is suggested that lymph nodes might serve as the point from which bacterial invasion may result. Heck (1955) observed large quantities of yeasts and bacteria on pork carcasses which had been delayed four hours prior to chilling and then held beyond the normal chilling period. Frazier (1958) relates other post-mortem microbial problems to the lack of concern for practicable asepsis during slaughtering and processing operations. More recent work reported by the A.M.I.F. (1960) has shown that freshly slaughtered and dressed carcasses have very few microorganisms on the surface, and the interior is virtually sterile except for the lymph nodes which may contain moderate numbers of bacteria. Baltzer and Wilson (1965) recently showed the presence of both aerobic and anaerobic flora on the rind side of pork carcasses during various processing sequences on Irish and Danish slaughtering lines. The ability to propagate within a muscle or on the exterior surface of a carcass has considerable bacteriological significance relevant to ham processing operations. To relate the awareness of the U.S.D.A. Meat Inspection Division (M.I.D.) to these implications, the requirements for pork slaughtering and processing establishments are quoted in part below (U.S.D.A., 1959):

> Slaughtering Operations (Hogs) - 53.6 (a) (1) (iii)
>
> Hog carcasses must be thoroughly washed, cleaned, and singed (when necessary) to remove all hair, scale, scurf, dirt and toenails on the slaughtering floor before any incision is made other than the stick wound. The forefeet when discarded in the slaughtering department need not be cleaned. Hog heads left on the carcass or sold intact must be thoroughly washed and flushed (nostrils, mouth and pharynx) and have ear tubes and eyelids removed.
>
> The routine post-mortem examination must consist in at least the following procedures:

1. Incise repeatedly and examine the two mandibular lymph glands.
2. Palpate the mediastinal and bronchial (right and left) lymph glands and palpate the lungs.
3. Examine and palpate the external surface of the heart.
4. Examine the liver and palpate the hepatic lymph glands.
5. Examine the spleen, stomach and intestines.
6. Palpate the mesenteric lymph glands.
7. Examine the exposed surfaces of the split carcass, the joints, the lining of the thoracic, abdominal, and pelvic cavities, and palpate the kidneys.

A number of workers have related various environmental influences on ante-mortem treatment of animals to deleterious affects due to subsequent bacterial invasion (Mossel and Ingram, 1955; Cosnett et al., 1956; and Hall et al., 1961). Kastenschmidt et al. (1964) investigated the influence of ante-mortem temperature and temperature fluctuations relative to explaining the nature and progression of metabolic porcine muscle constituents at the time of death as well as during subsequent post-mortem glycolysis. Saffle and Cole (1960) indicated that differences among pH determinations of porcine contractile tissue for different fasting periods were not significant for fresh ham samples. These workers reported that the range in pH for the fresh ham tissue was 5.3 to 5.4 after 24 hour chill. Briskey et al. (1962) indicated that most semitendinous porcine muscles approach the initial (onset) phase of rigor within 60-90 minutes of death when the normal internal temperature of the ham is 35-40°C. Bendall et al. (1963) showed that swine could be divided on the basis of post-mortem changes at constant temperature into two groups. They characterized one group by the fact that it underwent a slow glycolytic change while the other group showed a more rapid change. The variation among animal species and between animals within a species in the course or rate of lactic acid formation might be caused by: a) differences between membrane resistance to

autolytic processes or to increasing hydrogen ion concentration, b) deviations in post-mortem release of calcium and other ions by muscle proteins, and c) differences between the rates of glycolytic adenosine-triphosphate (ATP) resynthesis and its breakdown. According to Partmann (1963), the break down of ATP and its resynthesis by the glycolytic cycle will normally take place until the depletion of the glycogen reserve or a pH value of 5.4 is reached. Briskey et al. (1962) showed that at increased temperatures (43°C compared to 37°C) both the onset phase and total time for completion of rigor were markedly reduced.

Briskey and Wismer-Pedersen (1961) suggested that in addition to chemical composition, the chilling rate of the individual muscle may also be an important factor in establishing a pH pattern and ultimate muscle structure. The early work of Callow (1947) relative to the effect of increased lactic acid on porcine tissue microstructure appears to hold some significance since the change from closed to open tissue structure was reported to be slowed down by cooling. According to this author, the production of lactic acid in the fibers causes them to shrink and exude fluid. Mossel and Ingram (1955) reported that microscopic examination of meat tissues revealed that the growth of incipient spoilage bacteria was primarily confined to the fluid between the tissue fibrils. Frazier (1958) implied that it is only after the completion of rigor mortis that much of the food substrate is made available to exogenous bacteria. Hamm (1959) reported that the pH of fresh muscle is directly related to water holding capacity (WHC) due to electrical charges of the protein and the attraction of water by polar groups.

Niven (1961) suggested that since the tissues of animals provide such an excellent bacteriological medium, prompt chilling is a means of retarding the

growth of most contaminating bacteria. A selective environment is thus created which allows the growth of only a few types of bacteria. Upadhyay and Stokes (1962), in studies on the growth of psychrophilic bacteria under both aerobic and anaerobic conditions, found that decreases in incubation temperatures increased the duration of the lag, exponential, stationary and death phases. Elimination of oxygen increased the lag period, allowed the cells to survive longer at low temperatures (below 20°C) and reduced the extent of growth. Mossel and Ingram (1955) categorically list Achromobacter, Pseudomonas, Flavobacterium and Micrococcus as the associative bacterial genera dominating when spoilage occurs under standard storage conditions for fresh meats. Although many investigations have been carried out with respect to the species of Pseudomonas which are predominant at refrigerated temperatures, relatively few have been conducted on their nutritional requirements under reduced oxygen tension. In the case of chilled pork, a strongly proteolytic strain of Pseudomonas fluorescens is often the dominant species (Mossel and Ingram, 1955). Ayres (1960) investigated the role of pseudomonads with respect to the production of off-odors and slime on fresh beef under refrigerated storage. It was established that pseudomonads were responsible for the production of slime at 10°C or lower temperatures, whereas the incidence of pseudomonads and micrococcus was approximately equal at 15°C or above. The cell numbers necessary for appearance of slime was found to be 6×10^6. Heather and Vanderzant (1957) studied the effects of time and temperature of incubation and of pH of the medium on psychrophilic pseudomonads. These workers reported that the psychrophiles (P. fluorescens, P. fragi, P. putrefaciens) subjected

to a sublethal heat treatment were more sensitive to the pH of plating media than the nonheated controls. Unheated cultures of P. fragi manifested typical growth at pH 5.0. Nonheated cultures of P. fluorescens and P. putrefaciens gave essentially the same count over a pH range of 6.0 to 9.0. Wodzinski and Frazier (1961) reported that P. fluorescens and Aerobacter aerogenes exhibited greater ability to initiate growth at low water activity (a_w) levels at decreased oxygen tension than under regular aerobic conditions. The effects of the decreased partial pressure of oxygen on the organism studied caused variation in its apparent lag phase and generation time according to the level of oxygen employed, temperature and water activity (a_w). These workers hypothesized from the results of their accumulative data on the moisture requirements of bacteria that low water activity could influence the sensitivity of certain organisms to a specific environmental inhibitor. Vanderzant (1957) and Camp and Vanderzant (1957) reported on the endo- and extracellular proteolytic enzymes of P. putrefaciens. In addition to the Pseudomonas-Achromobacter group Jensen and Hess (1941) isolated Clostridium, Bacillus, Proteus, micrococci and diphtheroids from fresh unchilled ham. In early work many organisms were undoubtedly identified as Achromobacter because they lacked pigments and would now, according to the 7th edition of Bergey's Manual (Breed et al., 1957), be classified as Pseudomonas on the basis of flagellation. In comparing the ante-mortem and post-mortem bacteriology of the ham they suggested that bacterial invasion of the ham tissue occurs during and after death. Lepovetsky et al. (1953) reported isolation of pseudomonads and streptococci from the musculature of beef rounds and characterized their proteolytic activities in order that their potential role in spoilage

could be evaluated. The pseudomonads isolated produced a fruity odor and did not elaborate a diffusable pigment.

According to Niven (1961), joint or bone "sours" are occasionally encountered within deep muscle masses and are generally attributed to lack of prompt, thorough cooling. Thus, anaerobic sporeformers, as well as certain Gram positive, facultative bacteria propagate within the tissue. Niven (1964) states that major problems exist with pork and veal in linking salmonellosis with the increasing incidence of salmonellae in human foodstuffs. He suggested that interior as well as surface contamination of such meats must be considered. A number of workers have confirmed the presence of salmonellae in lymph nodes, edible organ meats and intestines of normal, healthy swine (Rubin et al., 1942; Galton et al., 1954; Hansen et al., 1964). Through such occurrences prior to slaughter, it would be reasonable to suspect the normal hog as a reservoir of infection. Niven (1964) implied that outbreaks of human salmonellosis have occurred in some instances from pork from animals which have undergone rigid ante-mortem and post-mortem veterinary inspection, thus indicating that frank subclinical infections may occur. According to Porter (1965), external stress (e.g. shipment for slaughter) on symptomless animals may break down their resistance with resulting multiplication of bacteria in the intestinal tract during the last hours or days before death. According to this author, this form of contamination can rapidly spread to the edible meats even if no systemic infection is indicated.

Another group of bacteria which must be considered among the total flora associated with the uncured ham are the sporeformers. Hessen and Riemann (1958)

acknowledged the importance of the raw material (fresh ham) with respect to its potential for being contaminated by sporeformers under routine handling conditions. According to Riemann (1963), the majority of bacteria on fresh pork are psychrophiles, and any sporeformers must originate from contamination during slaughter, handling or storage. This author implied that only those spores that did not germinate in the fresh meat tissue or were present as extrinsic, in-process contamination would be expected to present possible problems due to spoilage. Burke et al. (1950), Strong et al. (1963) and Hall and Angelotti (1965) have indicated that frequently the level of contamination by anaerobic spores is too low to be detected by means other than enrichment. Hall and Angelotti (1965) reported a 37 percent incidence of Clostridium perfringens in fresh pork cuts using an enrichment technique. The report of Steinkraus and Ayres (1964) that an average of less than 0.18 putrefactive anaerobic spores were present per gram of fresh pork trimmings analyzed by a fortified thioglycollate enrichment scheme indicates the sensitivity of the enrichment technique. Frank (1963) discussed the inadequacy of generalizations regarding the ability of sporeforming bacteria to propagate in cured meats because of the wide environmental parameters.

Due to the fact that the A.M.I.F. (1960) has established that none of the pathogenic bacteria associated with meat can grow at temperatures below 5°C, and that they normally grow very slowly, if at all, below 10°C in fresh meat, major concern by the industry rests with the conventionally adopted post-chill treatment for controlling problems of bacteriological origin. However, the relative margin of safety of this practice in providing the consumer with a safe, wholesome product is questioned.

Influences of Curing on Bacterial Activity in Ham

Coupled with the ordinary physical and biochemical changes that occur in fresh meat tissue are the effects of the variety of curing processes in practice. The latter certainly must also influence the bacteriology of the cured ham.

The early work reported by Callow (1947) on the selective effect of curing salts on the growth of microorganisms is still valid. According to the A.M.I.F. (1960), the influence of sodium chloride (NaCl) on the actual curing operation remains without question the most important single ingredient in the modern cure formulae. The physical and chemical characteristics of NaCl have been reported by Wistreich et al. (1959; 1960) as they are affected by time, temperature and solution concentration in the curing of ham. By application of surface-response methodology, the optimum salt level has been based upon a salt to sugar ratio of 2.5% sodium chloride to 1% sucrose as reported by Pearson et al. (1962) for the mild flavored "quick" cured ham. According to the A.M.I.F. (1960), salt inhibits the growth of most bacteria through the action of plasmolysis. Osmotic pressure increase rather than the chemical properties of the solute (NaCl) appear to account for the inhibitive effects within the tissue fibrils. Mossel and Kuijk (1955) implied that the inhibitive effect on bacteria is also influenced greatly by the decrease in the equilibrium relative humidity (ERH) value of the specific substrate. This concept is well supported in the advancement of a quicker technique for determining ERH of foods based upon known inhibitive sodium chloride concentrations for various food spoilage organisms. Frazier (1958) explained available moisture as it affects bacteria in foods and indicated that a_w would be in equilibrium

with the relative humidity of the atmosphere about a food. He stated that "the a_w for pure water would be 1.00, and for a 1.0 molal solution of a nonionizing solute the a_w would be 0.9823." Elliott and Michener (1965) define a_w "as P/P_o, where P and P_o are vapor pressures of the solution or system under consideration and of pure water, respectively. $P/P_o \times 100$ is also the relative humidity (RH) of the atmosphere in equilibrium with the solution." Observations reported by Riemann (1963) support the thesis that inhibition by NaCl in the concentrations normally employed in curing ham is due to the effect on a_w. According to Riemann (1963) the a_w in a ready-to-eat ham is 0.96-0.97 and is not sufficiently low to preclude the growth of sporeforming bacilli. The following data were used by Riemann to show the relationship of a_w-brine concentration:

a_w	Brine Concentration (Grams NaCl/100 g NaCl + HOH)	
0.93-0.94	11.0-9.7	Limiting for growth
0.96-0.98	6.7-3.5	Limiting for spore germination

According to Hamm (1959), the addition of NaCl to meat tissues increases the water-holding capacity (WHC). This implies the ability for a substrate to hold fast to its own or added water during the application of any physical force such as grinding, pressing or heating (Hamm, 1960). Hamm (1959) and Wismer-Pedersen (1960) showed that a good correlation existed between WHC of meat and the amount of curing brine absorbed. Jay (1965) noted a definite relationship between WHC of fresh beef and the bacterial quality of the tissues. Deatherage and Hamm (1960) showed that quick freezing with subsequent thawing of the tissue while in rigor had a small effect on the WHC. Weiner (1964) reported

significantly lower cooking losses and drip losses from hams cut within 1 hour of slaughter and injected with curing brine compared to the control hams which were pumped after conventional chilling. He postulated that the decrease was attributable to the reaction of the curing ingredients with the pre-rigor tissue. The degree of mobility and binding of natural meat electrolytes following the addition of NaCl and polyphosphates in brine concentrations commonly employed by the industry are discussed by Berman and Swift (1964).

The bacteriological aspects of the nitrate-nitrite mixture in terms of its antibacterial activity are discussed by Shank et al. (1962). These workers demonstrated that the pH of the environment has a pronounced effect on the bacteriostatic action of nitrite. It was shown that at 5.6 - 5.8, nitrite exerts a distinct bacteriostatic action; at pH 5.3 and below, nitrite disappears without any apparent activity. It was suggested by these workers that the formation of nitrous acid, via a pH-dependent mechanism, is responsible for the quantitative as well as qualitative changes in the bacterial flora in cured meat.

In the curing process, the smokehouse serves three important functions: (1) drying, (2) smoking and (3) cooking. All directly or indirectly affect the bacterial flora of the product. The chemical components of the wood smoke are known to impart preservative action through the varying amounts of high-boiling organic compounds of a phenolic type (aldehydes, ketones) and the lesser amounts of methyl alcohol, formic and acetic acids (A.M.I.F., 1960). It is implied from an excellent review by Draudt (1963) that smoking acts as a complementary preservative principally through the residual bactericidal effects of the smoke particulate. In addition, the heating in combination with low humidity and forced

air circulation causes a considerable amount of previously injected water (aqueous brine) to be evaporated from the product. Lowe (1955) reviewed the science of meat cookery and hypothesized that since the fat molecule is large, infiltration of fat during cooking is slight because the meat fibrils shrink and water with various salts and other soluble constituents is forced from the interior. The amount of moisture lost increases with increasing temperature, except in a range from 55°C to 65°C. Observations by Hamm (1959) revealed that pH of meat shows a relatively uniform increase with increased temperatures up to 75°C to 80°C. Saffle and Cole (1960) reported the pH range for cured and smoked ham was 5.4 to 5.5. According to Parker (1952), ready-to-eat hams are removed from the smokehouse after they have been maintained at a minimum internal temperature of 60°C for not less than 30 minutes and then cooled to an internal temperature of 7°C. This usually is accomplished within 8 hours. The hams were "tenderized" by the heat denaturation of the muscle proteins; however, oxygen was driven off, and due to the naturally low redox potential of the substrate, an anaerobic environment is established (Mossel and Ingram, 1955). Consequently, it has been shown that the majority of the bacterial flora capable of growing in cured, smoked and heat processed (tenderized) meats are largely Gram positive sporeformers and certain thermoduric cocci (Hessen and Riemann, 1958; Greenberg and Silliker, 1961; Riemann, 1963). Riemann (1963) characterized the Bacillus strains isolated from canned cured meat and pointed out the fact that some could multiply under anaerobic conditions. He stated that "ninety percent or more of Bacillus species isolated from canned cured meat could multiply in the presence of 10 percent NaCl (10 g. NaCl/100 ml HOH), and . . . .were not inhibited at pH 5.0." This writer

indicated that the majority of clostridia isolated from canned cured meats belong to the putrefactive group (C. sporogenes and similar species). Mundt et al. (1954) found that C. sporogenes germinated in meat held at 4.4°C but that vegetative growth was inhibited. Curran and Pallansch (1963) have also shown incipient germination of Bacillus spores at sub-minimal growth temperatures. The heat resistance of spores and vegetative cells found on hams prior to and after the processing of semi-perishable hams is discussed by Brown et al. (1960). Studies by Silliker (1959) have shown that putrefactive anaerobes can survive and sometimes propagate in the presence of appreciable concentrations of normal curing ingredients. Drake et al. (1960) implicated Streptococcus faecium as the lactic acid organism most likely to survive the processing treatment given perishable canned hams. Greenberg and Silliker (1961) implied that the longer cured pork products are cooked in excess of 65°C during heat processing, the greater will be the synergistic enterococci-killing effect of the nitrite.

Public Health and Product Quality Aspects of Cured Ready-to-Eat Ham

The development of the mild cured, ready-to-eat ham is indicative of a modern food product evolving from an intensely complex industry through a multitude of specialized processes. Despite the numerous scientific advancements, modernized merchandising, and critical quality control programs, the product remains quite vulnerable to many influences that can favorably or unfavorably affect its stability, organoleptic attributes, and hygienic value. Meat hygiene as defined by Edelmann et al. (1945) plays a vital role in preventive medicine

by guarding against the transmission of pathogenic microorganisms through diseased meats or meat of unsound quality. It is well known that specific bacterial associations and related problems normally will develop with any given food processing operation (Mossel and Ingram, 1955). According to the American Meat Institute Foundation (A.M.I.F., 1960), one of the most common meat products involved with the occurrence of staphylococcal food poisoning is cured ham. An excellent discussion relative to the etiology and epidemiological importance of this group of bacteria in cured meats is given. According to Niven (1961), ham must remain within the temperature range of 10° to 46°C for a minimum of four hours for the production of sufficient enterotoxin to cause illness. Heating the tissue to the maximum temperature consistent with edibility will not inactivate the toxin. Numerous reports in the literature (Dauer, 1961; Jay, 1962; Casman et al., 1963) reflect the various and indefinite sources of the pathogenic staphylococci occurring in the various forms of ham—including the "tenderized" product. Some of the classic characteristics of pathogenic staphylococci found in meats are discussed by Jay (1962). Staphylococcus aureus (Bergey's Manual, 7th ed.) has been found to produce a variety of active agents such as hemolysins, coagulase, hyaluronidase, penicillianase, lecithinase, and fibrinolysin as well as enterotoxin. One or more of these products may be directly associated with pathogenicity. Bergey's Manual (Breed et al., 1957) makes the distinction between irregular mass-forming cocci by placing all facultative species capable of utilizing glucose anaerobically in the genus Staphylococcus, and those species incapable of anaerobic growth in the genus Micrococcus. The three most important characteristics currently associated with the pathogenic strains of Staphylococcus aureus

are coagulase production, mannitol fermentation, and pigmentation (Jay, 1962; Jones et al., 1963; Lewis and Angelotti, 1964). A search of the literature indicated that a number of workers have attempted to relate the effect of various environments as well as inhibitory effects by concomitant flora on the biological activity of staphylococci in meats (Lechowich et al., 1956; Graves and Frazier, 1963; Peterson et al., 1964). Silliker et al. (1962) concluded from their heat resistance studies of enterotoxigenic strains of Staphylococcus aureus that conventional heat processing given to smoked and fully cooked hams is sufficient to eliminate any reasonable number of staphylococci. These workers implied that since the fresh tissue seldom contains greater than a few thousand staphylococci per gram, consideration of higher processing temperatures in unwarranted. Thus smoking (cooking) a ham to an internal temperature of 55 to 60°C is sufficient to preclude the growth of all staphylococci and salmonellae that might have been in it (A.M.I.F., 1960). Dunker et al. (1953) reported that of the bacteria found in fresh processed ready-to-eat hams the majority were nonpathogenic micrococci. Silliker et al. (1962) implied that enterococci and micrococci have a higher order of heat resistance than staphylococci. Angelotti et al. (1961) studied the time-temperature effects on non-sporing bacteria and indicated that the end-points of survival-kill at all test temperatures in ham salad were less than for other foods studied.

Consequently, from the standpoint of ham processing, it would appear that the various curing ingredients as well as the minimum temperature treatments permitted under federal inspection (U.S.D.A., 1962) are effective in precluding the survival of non-spore producing pathogens. However, a search of the literature

reveals that in most incidents involving meat-borne pathogens (cured ham), contamination invariably resulted from mishandling of the product after processing by the food handler or consumer (Angelotti et al., 1961; Dauer, 1961; Public Health Service, 1962).

Food poisoning incidents involving anaerobic and facultative anaerobic sporeforming bacilli in meats are reported by Dauer (1961) and Niven (1961). According to these writers, food poisoning episodes by C. perfringens and B. cereus are becoming increasingly common and have been demonstrated only during the past decade in the United States. This fact, however, may be attributed more to recognition than to lack of previous incidence. Hobbs (1965) attributes the well known heat resistance of types A and F spores of C. welchii (C. perfringens) as the most important factor with respect to the increasing incidence of acute gastro-enteritis. Hobbs (as reported by Appleman, 1957), asserted that it is essential to measure total aerobic as well as anaerobic flora since C. perfringens poisoning may take place in the presence of low aerobic and high anaerobic counts. Investigations implicate this anaerobe in pork processing operations not only in Great Britain, where it was first described, but in many other parts of the world (Ingram, 1952; Hall et al., 1961; Baltzer and Wilson, 1965). Lewis and Angelotti (1964) and Gibbs and Freame (1965) have reviewed rather thoroughly the methods for isolation and enumeration of clostridia in foods. Differentiation based upon the blackening reaction (FeS) given by most clostridia in media containing sulfite and iron is utilized almost exclusively. Frank (1963) reviewed the heat resistance characteristics of the sporeforming bacilli common to meats and meat products. He indicated that germination is a gradual process,

and that spores in the initial phases of germination had a reduced heat resistance. However, he also pointed out that in addition to the lethal effects normally associated with cooking, heat above the growth range of some sporulating bacilli can actually stimulate germination. The majority of these bacilli are reported to have optimum growth temperatures between 35° and 37°C (Breed et al., 1957). Burke et al. (1950) showed that the MPN technique was a most satisfactory method for determining spore incidence in commercial pork products. Cochran (1950) reviewed the statistical basis of the MPN method and discussed the planning of appropriate dilution series. Steinkraus and Ayres (1964) and Gibbs and Freame (1965) have substantiated the fact that MPN counts are more sensitive for counting small numbers of putrefactive anaerobic spores in meat and other foods. According to Niven (1961), semi-perishable canned hams have enjoyed a remarkable safety record and botulism is a rare occurrence in cured meats. Pivnich and Bird (1965) described toxinogenesis by C. botulinum types A and E in ready-to-eat ham products packaged in air-permeable and in air-impermeable plastic. By artifical contamination, these workers were able to show that toxinogenesis was dependent upon the type of C. botulinum, concentration of spores at the time of inoculation, time and temperature of incubation, and type of meat product. Type of packaging affected spoilage but not toxinogenesis. The meat products evaluated spoiled more readily in air-permeable than in air-impermeable plastics. These workers pointed out that since toxinogenesis without spoilage was possible, the consumption of toxic, ready-to-eat meat thus existed as a hypothetical hazard. They attributed the perfect safety record of meat products packaged in air-impermeable plastic to (1) a very low level of anaerobic spores in meat (Burke et al., 1950; Riemann, 1963;

Steinkraus and Ayres, 1964), (2) the addition of NaCl with the availability of nitrite, and (3) storage of the cooked product at refrigerated temperatures. Pivnick and Barnett (1965) showed that toxinogenesis in artifically contaminated cooked ham (10,000 spores/g.) could be delayed by increasing the concentration of salt and decreasing the temperature. These workers demonstrated that at 20°C ham containing 2.1% salt required more than five but less than ten days to become toxic. At 15°C, toxinogenesis did not occur within one month with salt concentrations between 2.1 and 3.4%. Dack (1964) has reviewed the U.S. Public Health Service's Morbidity and Mortality Weekly Report(s) from 1950 through 1963 and reported only one case involving a cured ready-to-eat pork product in which one death resulted from the consumption of pickled pigs' feet. An untyped botulism toxin was detected in the glass packed product.

Niven (1961) implied that the majority of interior discolorations which are associated with cured pork are due to underprocessing. According to the A.M.I.F. (1960) interior discoloration may result from direct chemical changes which may or may not be of bacterial origin. The bacteria implicated in cured meats are those organisms (catalase negative) capable of producing hydrogen peroxide (H_2O_2) which oxidizes the porphyrin ring to cholemyoglobin (green). Under anaerobic environment such organisms respire without the production of H_2O_2. Zatovil (1963) explained the catalytic effect of nitrite in bacterial "greening" in terms of a disintergration effect by hydroxyl radicals from the decomposition of pernitrous acid (auto-oxidation via H_2O_2).

Even though bacterial poisoning and food spoilage problems appear to be

under control in conventionally processed hams, the trend toward milder processing lends serious grounds for questioning the actual margin of safety involved.

Recent work by Pulliam and Kelley (1965) showed that coagulase-positive staphylococci were capable of surviving hot processing techniques utilized to cure whole, bone-in hams which were artery and stitch pumped prior to undergoing a 14 day curing period and then smoked. These workers implied that hot processing may allow for faster growth by potential food poisoning staphylococci which were isolated from the tissue of one hot processed ham out of 15 surveyed.

With the foregoing observations reviewed in the literature, investigations were undertaken to enumerate and identify the associative bacteria within the deep, subsurface tissue from uncured and cured conventional and rapid processed, pre-rigor ham. In addition, observations relative to the effect on product quality and hazard(s) to public health were investigated.

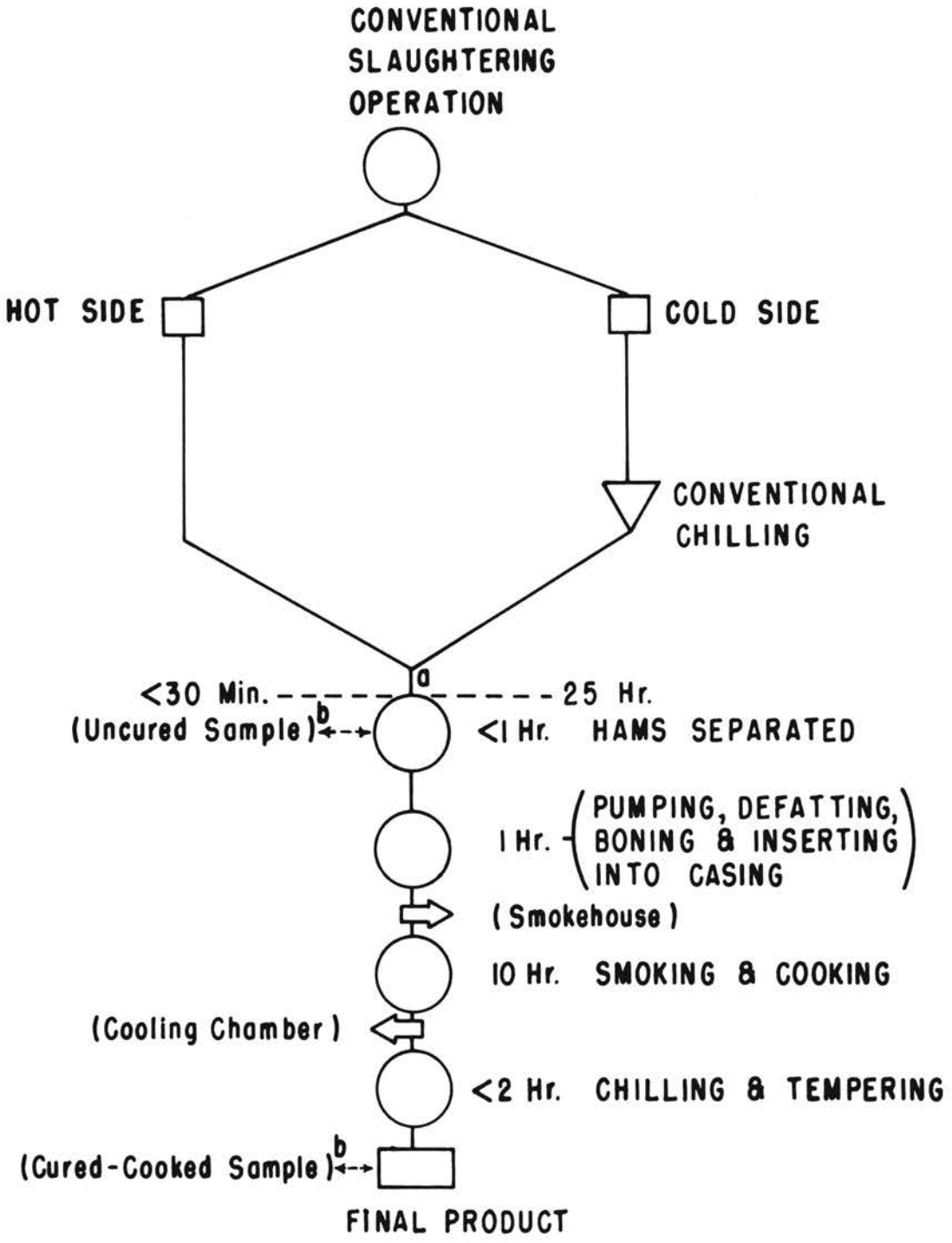
METHODS AND PROCEDURE

Source of Meat

Twenty lightweight barrows of similar breeding, feeding, and management were selected from the Experiment Station herd and assigned to a slaughtering schedule of approximately four weeks duration. The pigs (3) were delivered to the abattoir holding room approximately 15 hours prior to each kill day and withheld from feed and water overnight. The animals were slaughtered by procedures established at the Oklahoma Station Meat Science Laboratory with no overt attempt by slaughtering personnel to employ special aseptic precautions other than those normally considered to be "standard for the trade." Following exsanguination, conventional scalding, dehairing, singeing and evisceration, the carcasses were split with each half being alternately assigned to either rapid or conventional treatments. Rapid treatment implies fabrication of the side (half) prior to normal chilling, whereas the conventional treatment included a 24 hour initial chilling period at 1.7°C. Cutting procedures utilized for separating the hams from their respective treatment sides were identical to those employed in the industry. No attempt was made to relate treatment effects to the previous history of the animals, since the paired ham experimental plan was followed and the right or left ham of each pair served as an untreated control for each carcass.

Ham Processing, pH, Residual Moisture and Salt Measurement

Routinely following the product flow diagram illustrated in Figure 1, all hams were stitch-pumped with a 60° salinometer brine composed of: salt, sucrose, dextrose, nitrate, nitrite, erythorbate, bicarbonate, and polyphosphates. The corresponding brine concentration was approximately 15 percent NaCl (Grams NaCl/100 ml NaCl + HOH). Required amounts of brine were prepared fresh daily from the commercially available curing ingredients with cold tap water in a manner consistent with approved meat inspection regulations of the U.S.D.A. (1962, 1964) and accepted industry practice. After the hams were skinned and the outside fat removed, they were weighed, sampled, muscle pH measured, and, by means of a percentage scale, injected to 110 percent of green weight. They were immediately boned, defatted, stuffed into individual casings (Fibrous Visking No. 11-smoke permeable), placed into molds and moved into the smokehouse. After a one hour initial drying period, smoking was commenced and continued for eight hours at 55°C. The smoke was generated from Georgia hardwood sawdust that had been dampened prior to ignition. After smoking, the hams were cooked in-place to an internal temperature of 60°C for a two hour period. The smoked and fully cooked hams were removed from the smokehouse and cooled rapidly in a Weber rapid cooling chamber. The air movement in the chamber was turbulent and shown by Smith et al. (1965), to have an effective approach velocity of 120 fpm. Temperature distribution and rate of cooling was monitored by means of thermocouple probes inserted at the geometric center of the ham, at one-half inch and at one inch from the center. The temperature of the cooling chamber air was varied from -23°C to-62°C with two groups of



a Time sequence equal beyond this point

b For bacteriological analyses

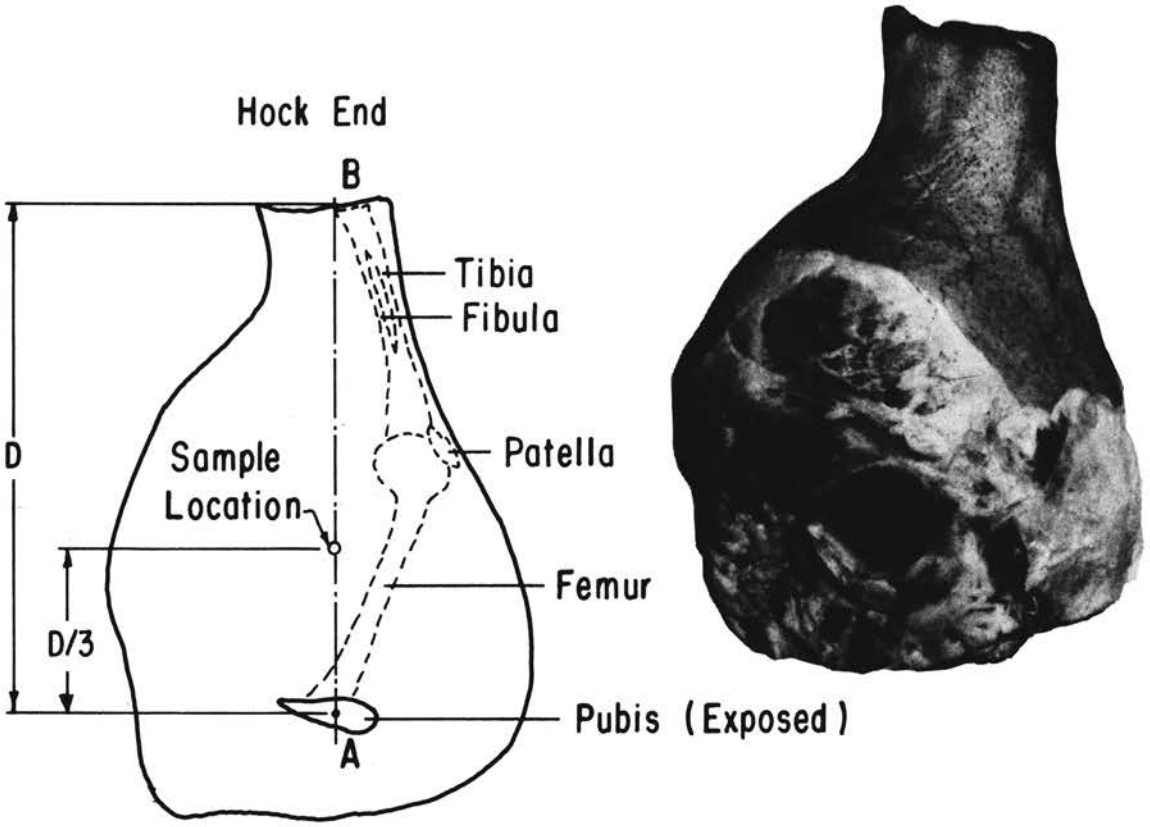
Figure 1. Product flow diagram.

coded hams. A 10 point potentiometer was used to record the changing internal temperature of each ham. Cooling time was adjusted so that when the internal temperature reached 10°C, the hams were removed from the cooling chamber to a chilling room maintained at the conventional meat holding temperature (1.7°C). When the cured and fully cooked hams had tempered to an average internal temperature of 7°C, terminal samples and pH measurements were taken. Ham tissue adjacent to the cored area was trimmed and subsequently ground to a paste consistency for percent moisture analysis (A.O.A.C., 1960). Aliquots from the ground samples were titrated with 0.1N silver nitrate by the method of Kamm (1964) for percent NaCl. All pH measurements were taken immediately following the removal of the samples at the points shown on Figure 1. The hydrogen ion concentration was measured by placing a standardized (pH 7.0 $\pm$ 0.01) single probe electrode from a line operated Beckman model 72 meter into the exposed deep muscle tissue. Means and standard deviations of the pH, salt concentration, and residual moisture were computed and statistically analyzed (Snedecor, 1956).

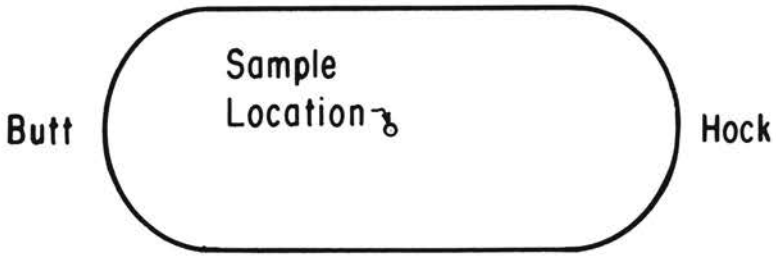
Sampling Technique

To establish the initial load and to subsequently evaluate processing effects on the bacterial flora of the processed tissue, bacteriological survey core samples were aseptically removed from treatment hams at the points indicated in Figure 1. The core samples were obtained by a mechanical boring device which was powered by a portable one-fourth inch, Black and Decker (Model U-400) electric drill. Prior to sampling, each borer was disassembled and the stainless steel components

physically cleaned. The plunger assembly and cutting tube was thoroughly coated with a light-covering of a nontoxic, high melting-point, silicone compound; reassembled and wrapped individually in lightweight kraft paper for sterilization. The sterile-wrapped borer was prepared for aseptic sampling by tearing off only the overwrapped portion from the shank end of the borer and firmly affixing the device to the electric drill by means of the "chuck" gear key. Just prior to coring, the cutting tube was extended and locked, the remaining "sterile-wrap" removed, and the sampling location heat seared with an electric searing iron. Each aseptically cored sample was removed from the borer by pushing the core into a sterile screw-top, glass jar. A code number reflecting the carcass number, treatment, type of sample (uncured or cured), and the date was placed on each sample jar and on a data collection sheet on which was recorded the corresponding pH value. The common sampling locations for the uncured and cured-cooked cores are illustrated in Figures 2a and 2b, respectively. All uncured samples were taken with the posterior portion of the shank (hock) pointing 180 degrees away from the sampler and with the exposed medial pubic bone oriented vertically in full view. From the mid-point of the pubic bone (point A) the edge of an 18 inch rule was aligned in such a manner to form a straightline distance (D) to a mid-point (B) on the hock. One-third the distance from A to B became the uncured sample location and allowed the bore to be inserted to the desired depth without difficulty. All cured-cooked samples were cored horizontal to the flat face of the molded boneless ham at the mid-point of the longest side. On days when cured-cooked samples were collected, the core samples were placed immediately under refrigeration (10°C) until all uncured (fresh) samples had been prepared for bacteriological plating. Average elapsed time for collection of the uncured sample until



a. Uncured Ham (Medial View)



b. Cured - Cooked Ham (Horizontal View)

Figure 2. Diagram showing sampling locations.

the aerobic platings were completed was approximately 30 minutes. In keeping with the daily slaughtering schedule, three hams from the rapid treatment and three of the conventionally chilled (paired) hams from the previous day were sampled.

Bacteriological Laboratory Techniques

Except for certain special media, all primary and differential media were prepared fresh from new stock of commercially available media according to the manufacturer's recommended directions. All media and reagents were prepared in deionized-distilled water and according to generally accepted procedures except where tap water or non-aqueous solutions were specified.

Pipettes were cleaned by submerging in a chromic acid solution with one "end-for-end" inversion after soaking for not less than one hour. Each pipette was withdrawn individually and thoroughly rinsed with tap water followed by three rinses with distilled water. Cleaned pipettes were then dryed prior to wrapping for sterilization. All other glassware was routinely cleaned by soaking in a trisodium phosphate detergent and brushing followed by copious rinsing in tap water and a single distilled water rinse. Xylene was regularly used as a pre-rinse for all equipment containing fatty material. When the need became apparent, the chromic acid treatment was applied to other glassware.

Whenever possible, sterilization of media was accomplished by autoclaving at 121°C for 15 minutes at 15 pounds pressure. All sampling equipment and other glassware requiring an overwrap for use in aseptic sampling or plating schemes were wrapped with lightweight kraft paper and marked with a time-temperature "sterile" indicator label prior to being autoclaved. After sterilization at 121° for 15 minutes

at 15 pounds pressure, all overwrapped metal and glass articles were dried at 105°C in an air-convection oven for 30 minutes and then removed to cool to room temperature before placing into storage drawers.

Photomicrographs were taken on Polaroid PolaPan Type 52 film. A Bausch & Lomb "Dynoptic" microscope with 97x oil immersion objective and 10x standard eyepiece as projecting ocular for a Leitz Aristophot camera was used. Illumination was by the Koehler critical method with an exposure time of 0.2 seconds.

Bacteriological Analyses

Two procedural lines of investigation are presented for the experimental work herein reported: (1) quantitative phase for enumerating both aerobic and anaerobic bacteria and (2) qualitative phase for identifying all bacteria isolated from aerobic and anaerobic culturing schemes. Each phase is sub-grouped into the separate methods and procedures utilized to study the isolated bacteria.

Aerobic Flora (Quantitative)

Each sample was analyzed on a total count basis by standard bacteriological pour plate methodology. Ham tissue homogenates were prepared by placing an eleven gram (inner) portion of the cored sample into a sterile glass Waring blendor jar containing 99 milliliters of phosphate-buffered saline diluent (Sulzbacher, 1953; Lewis and Angelotti, 1964). Samples were blended for two and one-half minutes at the low speed setting (approx. 12,000 rpm) on a standard laboratory model, two-speed Waring blendor. Prior to pipetting the required sample aliquots for platings, each dilution blank containing the homogenate with glass beads was shaken vigorously

for two minutes. Quadruplicate platings at decimal dilutions of 10^{-2} to 10^{-4} using Bacto tryptone glucose beef extract agar (TGEA) were made as well as replicate inoculations to certain qualitative media (See Qualitative section). Duplicate sets of TGEA plates were incubated at 37°C, with the remaining set being incubated at 15°C. After three days at 37°C or seven days at 15°C, appropriate plates were counted and the number of aerobic bacteria per gram of original sample computed. Those TGEA plates indicating minimal or no growth were re-incubated for 48 hours prior to disposal. Coliform bacteria (Escherichia and Aerobacter species) were quantitated by plating in Bacto violet red bile agar (VRBA) at dilutions of 10^{-1} to 10^{-3} and incubated at 37°C for 24 hours. Only the dark red colonies at least 0.5 mm. in diameter were counted (Lewis and Angelotti, 1964). Confirmation of actual coliform colonies was accomplished by means of indole, methyl red, Voges-Proskauer, and citrate (IMViC) reactions. Artifical contamination of controlled samples with known coliform bacteria was utilized as a positive check on the selectivity of the VRBA media and performance of the IMViC tests.

Aerobic Flora (Qualitative)

Replicate inoculations with 0.1 milliliter aliquots of the 1:10 original homogenate were spread over the surface of duplicate plates of Bacto Staphylococcus Medium 110 and of nile blue sulfate fat agar (NBSFA) by means of a sterile, bent, glass rod. Each plate was incubated for three days at 37°C or seven days at 15°C. The NBSFA medium was prepared using a modification of the method of Sulzbacher (1951) as described in Appendix A. After the TGEA plates had been counted, representative colonies were selected for isolation from the surface of the TGEA, Bacto

110, and NBSFA plates. The cultures were subsequently tested for purity and screened to eliminate duplication by the following tests: (1) Gram stain, (2) catalase production, (3) relation to oxygen, (4) motility, and (5) oxidase reaction. These tests and observations were performed routinely on all selected isolates. Upon the final selection of representative pure cultures, isolates were grown on slants of TGEA in 15 x 150 mm. screw cap test tubes. A standard system was devised for recording certain morphological, physiological, and cultural characteristics based upon a slight modification of the Descriptive Chart published by the Society of American Bacteriologists (1957) and incorporating the respective sample codes from both rapid and conventionally processed ham samples. Appropriate supplemental biochemical tests were selected for each group of isolates based upon the classification of bacterial genera as given in the 7th edition of Bergey's Manual (Breed et al., 1957). The qualitative aerobic flora were grouped as follows:

Group I - Non-sporing rods.
Group II - Catalase positive sporing rods.
Group III - Gram positive or Gram variable cocci.
Group IV - Gram negative rods.

Applicable biochemical and physiological reactions were utilized to characterize and identify the isolates in each group. The following tests were run on all isolates in Groups I and II: (1) acetylmethylcarbinol, (2) H_2S, (3) indole, (4) nitrate reduction, (5) citrate, (6) urea, (7) anaerobic growth and gas formation in glucose and nitrate broth (Methods of Smith et al., 1952), (8) aerobic fermentation of glucose, lactose, sucrose, mannitol, and glycerol (Difco, 1953), and the hydrolysis of casein, gelatin, and starch as described by Smith et al. (1952). The litmus milk reactions were recorded for all groups. All cultures in Groups I and II were further tested for

the ability to produce spores by separately inoculating two loopfuls from 12 and 24 hour old cultures grown on fortified TGEA (Appendix A) into screw cap tubes of sterile phosphate-buffered saline water (Sulzbacher, 1953; Lewis and Angelotti, 1964) containing glass beads. The tubes were thoroughly agitated by shaking and then 1 milliliter aliquots inoculated into tubes containing 10 milliliters of sterile Bacto nutrient broth and pasteurized for 10 minutes in a thermostatically controlled water bath held at 80°C. A blank tube with a thermometer (checked for accuracy) inserted through a No. 2, one-hole rubber stopper, into the nutrient broth was used to control the pasteurization treatment. After the cultures had been heated for the prescribed period they were cooled immediately in an ice bath and then placed in an incubator held at 37°C. Positive cultures were then re-inoculated onto TGEA slants in 15 x 150 mm. screw cap test tubes. Spore stains were prepared from these cultures after a 24 hour incubation period. The heat fixed smears were stained by the Bartholomew-Mittwer "cold" method as described by Lewis and Angelotti (1964).

The bacteria in Group III (Gram positive and Gram variable cocci) were further subgrouped according to morphology exhibited by Gram stains into Sub-Group A which contained all irregular mass forming cocci. Sub-Group B contained all other coccus forms within Group III. The following tests were accomplished on all isolates in Group III, Sub-Group A: (1) catalase, (2) relation to oxygen, (3) nitrate reduction, (4) hydrolysis of gelatin, (5) bound coagulase, and (6) hemolytic activity on sheep and rabbit red blood cell agar plates at a concentration of 4 percent blood (Appendix A). Anaerobic utilization of mannitol and glucose was determined in Bacto phenol red base broth with 1 percent mannitol and glucose, respectively.

Anaerobiosis was obtained using an anaerobic incubator (National Appliance Company) which was thermostatically controlled at 37°C. The cultures were flushed twice with hydrogen followed with the final gas phase consisting of a purified mixture of 90 percent nitrogen and 10 percent carbon dioxide (Matheson Company, Inc.). A commercial oxidation-reduction indicator agar (Difco) was utilized to test anaerobiosis throughout the culturing period. In addition to the various biochemical tests, the ratio of pigmented to nonpigmented colonies was noted for the cultures isolated from the Bacto Staphylococcus Medium 110.

With the exception of the bound coagulase test, all of the above tests were performed with the bacteria contained in Group III, Sub-Group B. In addition, all cultures in this sub-group were inoculated into Bacto-KF streptococcus broth. These tubes were incubated at 37°C for 24 to 48 hours.

Most of aerobic Gram negative rods contained in Group IV were isolated via the alternate enrichment scheme described by Lewis and Angelotti (1964). The only major deviation from this recommended scheme was the elimination of the serological procedures, since any suspected salmonellae isolates would be forwarded to the Communicable Disease Center (Public Health Service) for confirmatory typing. The streak plate technique was substituted for the recommended "hockey stick" technique in transferring positive enrichment broth cultures to the brilliant green sulfadiazine agar plates. The following biochemical characterizations were made with isolates from the enrichment broth as well as those obtained from the various coliform and TGEA plates: (1) oxidase, (2) urea, (3) H_2S (BACTO-SIM Agar), (4) KCN, (5) decarboxylation of arginine and lysine, (6) ninhydrin,

(7) fermentation of glucose, lactose, sucrose, maltose, and dulcitol, and (8) the IMViC reactions. Motility was only observed in the Bacto SIM medium with no detailed study of the flagellation of the organisms. Proteolytic characteristics were evaluated on gelatin and casein agar plates as described by Smith et al. (1952).

Anaerobic Flora (Quantitative)

Anaerobic bacteria were quantitated using the MPN technique as outlined by Cochran (1950) for estimating total bacterial numbers. The procedures generally followed those employed by Steinkraus and Ayres (1964) with the exception of choice of enrichment media and dilutions. Enumeration followed pasteurization at 60°C for 55 minutes (Greenberg, 1965) of the remaining portion of each homogenized sample from which total aerobe and coliform counts had been plated. After pasteurization, MPN counts with Bacto fluid thioglycollate medium modified with a sulfide detecting agent (0.02 percent ferrous ammonium sulfate) were seeded in 5-tube replicates using dilutions corresponding to volumes of 10, 1.0, and 0.1 milliliters of the original 1:10 meat-saline homogenate. All tubed media were steamed for 15 minutes and rapidly cooled prior to seeding in order to expel residual oxygen. The tubes were incubated 5 days at 37°C. All tubes showing visual indication of growth were subcultured for the qualitative phase of the survey. Only those tubes having a black precipitate (FeS) and producing a putrefactive odor were recorded as positive. The most probable number of anaerobic spores was estimated by referring to MPN tables from Standard Methods for the Examination of Water and Wastewater (1960). In addition to the MPN counts, duplicate pour plates were made with Bacto-SPS (Sulfite-Polymyxin-Sulfadiazine) agar as recommended by Lewis and

Angelotti (1964) for an additional check for Clostridium perfringens. Anaerobiosis was obtained as described previously with an atmosphere of 90 percent N_2 and 10 percent CO_2 in the anaerobic incubator. All anaerobic plates were checked after 24 hours incubation at 37°C. Negative plates were re-incubated for 24 hour prior to disposal.

Anaerobic Flora (Qualitative)

Growth from positive MPN tubes was transferred onto duplicate plates of 4 percent sheep blood and McClung-Toabe (1947) egg yolk agar. Both sets of plates were incubated at 37°C in the anaerobic incubator under an atmosphere of 90 percent N_2 and 10 percent CO_2. After 24 hours incubation, the blood plates were observed for hemolytic colonies and the egg plates for lecithinase-producing colonies. The following initial screening tests were run on each culture: (1) Gram stain, (2) catalase, (3) relation to oxygen, and type of reaction in litmus milk. Further identification of the sporulating anaerobes followed along the lines described by Spray (1936). The other media and techniques utilized were those which have become routine for positive identification of anaerobic organisms (Merchant and Packer, 1962; Lewis and Angelotti, 1964; Gibbs and Freame, 1965). Spore stains were prepared by the Bartholomew-Mittwer "cold" method as described by Lewis and Angelotti (1964).

RESULTS AND DISCUSSION

Bacterial Flora and Environment (Quantitative)

Bacterial numbers are based upon the quantitation of 19 pairs of ham from a total of 20. The pair of hams removed had extremely high bacterial counts which was attributable to a developing bacteremia and therefore was not included in the statistical treatment of the data. It was felt that emphasis should be given to the feasibility of similar high counts occurring under practical conditions wherein no visable disease state exists. There were no hot processed cured ham samples exceeding 109 bacteria per gram of sample, whereas 2 cured control hams yielded samples with high counts (Appendix B, 305 and 1,327 bacteria per gram) as summarized in Table I. Categorically, 68 percent (13) of the cured hot processed hams gave samples from which no colonies could be detected or extremely low numbers appeared (0 to 9 per plate). In the cured conventional processed hams only 53 percent (10) of the samples were in this category. Otherwise, treatment and control hams appeared quantitatively comparable at the mesophilic incubation range (37°C). Incubation at the psychrophilic range (15°C) yielded erratic low counts in 38 samples. Enumeration of the remaining samples resulted in less than one psychrophile per gram. An explanation for the high ratio of aerobic mesophilic counts to aerobic psychrophilic counts is easily advanced for the hot treatment; however, in the cold treatment no apparent reason exists for the erratic low

counts. Since the flesh of freshly slaughtered healthy meat animals is generally considered to be sterile, it would possibly be an expression of the inadequacy of the plating methodology. However, incubation at 37°C indicated that a detectable incidence of bacteria existed in all of the conventionally treated hams. Therefore, while less than 300 bacteria per gram were detected in the uncured tissue from both treatments (Table II) in which the samples were incubated at 37°C, one must consider that in mixed bacterial populations, real differences in resistance to chemicals, pH, and heat can be expected. The presence of the aerobic and facultative anaerobic bacteria in the fresh (uncured), hot processed tissue may be attributable to the post-mortem depletion of leucocytes, complement, and antibodies when muscle pH has not reached an inhibitory concentration.

TABLE I

INCIDENCE OF MESOPHILIC BACTERIA IN HOT AND COLD PROCESSED HAMS[a]

Colonies/Sample	Number of Hams			
	Hot		Cold	
	Uncured	Cured	Uncured	Cured
Aerobic:				
> 300	(3)[b] 2	-0-	(6) 5	2
10 to 300	11	6	8	7
< 10	6	9	5	7
Undetected	-0-	4	1	3
Total	19	19	19	19
Anaerobic:				
> 5	-0-	-0-	-0-	-0-
1 to 5	-0-	1(4)[c]	-0-	-0-
< 1	1(2)	-0-	1(2)	1(2)
Undetected	18	18	18	18
Total	19	19	19	19

[a] A total of 38 hams analyzed.
[b] One pair of hams removed from statistical treatment of the data.
[c] Incidence per sample by the MPN method given in parenthesis.

from animals of uniform breeding, feeding, and weight range, were slaughtered over a four week schedule. Although the animals were exsanguinated in the same abattoir by the same equipment and personnel, they responded individually at death to various stresses as well as to uncontrollable fluctuations in humidity and temperature. The high standard deviations (Table II) for the comparative aerobic flora (uncured hot, uncured cold, cured cold) are attributed to variation in the initial bacterial load of individual fresh hams as well as to the differing growth environments common to the two treatments. Also indicated, are the inherent enumeration problems when sampling low bacterial populations.

According to the A.M.I.F. (1960) most commercial curing operations maintain a curing temperature of 3°C ± 1°C because temperatures above 4°C may permit spoilage by the rapid growth of bacteria. Refrigeration below 2°C greatly delays curing time and therefore is not practiced by the trade. Thus it is apparent that the "ideal" curing temperature is in fact a compromise between rate of cure and rate of bacterial development. Unpublished data (Henrickson et al., 1965) from 20 pork sides weighing approximately 34 Kg each showed that the internal temperature of the whole hot-processed hams ranged from 39° to 43°C without chilling. The internal temperature of hams undergoing conventional chilling for 24 hours ranged from 7° to 9°C at the time of separation (Figure 1). Briskey and Wismer-Pedersen (1961) found that the rate of heat removal from porcine muscles undergoing rigor was of great importance in determining the total time for completion of rigor mortis. From the comparison of the initial mean pH values shown in Table III (uncured), the differences due to the time-temperature effects on anaerobic

muscle glycolysis are apparent for the two treatments. However, a comparison of the final mean pH and residual moisture values indicate that the ultimate pH was not affected by the rapid, hot processing treatment. The selective effect of pH on bacteria is well known. Likewise, bacterial cells or spores are known to be most heat-

TABLE III

RESIDUAL MOISTURE, SALT AND pH VALUES[a]

	Sample Treatment			
	Hot		Cold	
	Uncured	Cured	Uncured	Cured
Moisture %	-----	65.75	-----	66.49
Std. Dev.	-----	3.01	-----	3.77
Salt %	-----	1.57	-----	1.37
Std. Dev.	-----	0.27	-----	0.38
pH	6.46	5.83	5.70	5.84
Std. Dev.	0.24	0.17	0.16	0.20

[a] N=19

resistant in a substrate that is at or near neutrality (Frazier, 1958). Also, low pH induces cellular fragility which could render the bacterial cell more liable to the synergistic effects of nitrite (Shank et al., 1962). In addition, the classic work by Callow (1947) relative to the effects of increased lactic acid formation on porcine tissue microstructure may also bear bacteriological significance.

The significant treatment effects on aerobic bacterial numbers as shown in Table II for cured hot (15.4) versus cold (107.8) indicates that the combined factors associated with hot processing (i.e., pre-rigor injection, higher muscle temperature, and rapidity of handling) were more effective in reducing the bacterial load. Since no differences in the percent residual moisture, NaCl, or pH (Table III) were found

in the finished product from both treatments, the combined factors associated with rapid, hot processing apparently are responsible for the greater reduction.

The low incidence of anaerobic spores estimated by the MPN technique are shown in Table I. The presence of anaerobic spores in the tissue of only four different ham samples out of the 38 surveyed is in agreement with the findings of Burke et al. (1950) and Steinkraus and Ayres (1964). All anaerobic SPS plates yielded negative results for the presence of any anaerobic spores. However, since four samples did yield MPN counts, a general level of less than 10 anaerobic spores per gram of sample is indicated (Strong et al., 1963; Hall and Angelotti, 1965). Shank et al. (1962) demonstrated the antibacterial attributes of nitrous acid on both vegetative cells and spores. Due to the accelerated handling of the hot-processed ham, the effects of pH, curing ingredients, and temperature appear critical with respect to logarithmic growth of germinated exogenous spores. Since mature bacterial spores are the type most likely to be encountered as environmental contaminants, it appears that rapid, hot processing would be equally effective in reducing in-process contamination. A number of investigators have shown that incipient spore germination by both aerobic and anaerobic sporeforming bacilli can occur at sub-minimal growth temperatures (Mundt et al., 1954; Curran and Pallansch, 1963). Therefore, the more rapid exposure of the ubiquitous spores to known lethal factors should favor their elimination. Completed germination normally requires 2 to 3 hours under optimal conditions and sporulation generally takes another 6 to 12 hours with a maximum at approximately 15 hours. Thus, the normal exponential development of clostridial cells would render them sensitive to the lethal effect of processing.

Quantitation of coliform bacteria in both treatments revealed an extremely low incidence. Uncured and cured samples from conventional and cured hot hams had less than one confirmed coliform per gram of sample. Fresh samples from the uncured hot processed hams had four confirmed coliforms per gram of tissue. However, the presence of these aerobic and facultative anaerobic bacilli in uncured hot processed hams is reasonable since they had longer exposure to elevated temperatures and muscle pH had not reached an inhibitory value at the time of sampling. Since this level was subsequently reduced to less than one coliform per gram by processing methods, no particular significance is attached to this group of bacteria.

Isolation and Classification of Aerobic Bacteria

More than 200 bacterial cultures were isolated from the various plating and enrichment media. Thirty eight different uncured ham samples, of which 19 had been obtained from rapid, hot processed hams and 19 from conventionally processed hams were surveyed. From these samples, 101 pure cultures were selected as being representative of those found in uncured pork tissue. A total of 80 cultures were initially isolated from the 38 cured-cooked ham samples. Subsequent platings, replatings, and purification of these isolates yielded 52 cultures as being representative of the flora associated with the rapid and conventionally processed hams. The distribution of the total associative aerobic bacteria is provided in Table IV.

Bacterial Flora Associated with Uncured Tissue

On the basis of the results obtained from various screening tests, morphology, and colony characteristics, the isolates were arranged into the four groups of genera

TABLE IV

SUMMARY OF BACTERIAL GENERA AND FREQUENCY OF ISOLATION[a]

	Frequency of Isolation						Total
	Hot			Cold			
AEROBIC:	Uncured	Cured	Sub Total	Uncured	Cured	Sub Total	
I. Rods; Gram + (non-sporing)							
Bacterium	3	2	5	-0-	-0-	-0-	5
Microbacterium	5	-0-	5	3	-0-	3	8
			10			3	
II. Rods; Gram + (sporing)							
Bacillus	2	10	12	3	7	10	22
III. Cocci; Gram +							
Gaffkya	2	-0-	2	-0-	-0-	-0-	2
Micrococcus	7	4	11	8	6	14	25
Staphylococcus	8	5	13	7	8	15	28
Streptococcus	6	2	8	1	3	4	12
Sarcinia	2	-0-	2	-0-	-0-	-0-	2
			36			33	
IV. Rods; Gram -							
Alcaligenes	2	1	3	1	-0-	1	4
Escherichia	5	-0-	5	5	-0-	5	10
Proteus	1	-0-	1	-0-	-0-	-0-	1
Pseudomonas	17	3	20	13	1	14	34
			29			20	
TOTAL AEROBIC	60	27	87	41	25	66	153

[a] A total of 38 hams analyzed.

shown in Table IV. Since the main purpose in plating the uncured tissue was to establish the initial product load, only those isolates occurring in more than 5 different samples were identified to species level in the taxonomic distribution. From Table IV the bacteria present in more than 5 different uncured treatment samples were the micrococci, staphylococci, streptococci, and pseudomonads. Little significance was attached to the prevalence of the other genera with the exception of the coliform bacteria. Escherichia coli is a recognized indicator organism for assessing the hygienic condition of most human foods. The coliforms were identified as predominately E. freundii on the basis of the results given in Table VI. However, since the low incidence was reduced to less than one confirmed coliform per gram by processing methods, no further significance is attached to this group of bacteria. Groups I and II were distinguished by means of laboratory pasteurization at 80°C for 10 minutes. Those test cultures which failed to survive this treatment were tentatively assigned to the non-sporing, Gram and catalase positive rod group. Cultures surviving the pasteurization treatment were placed into the sporulating, Gram and catalase positive group. On the basis of morphological, cultural, and biochemical tests, further characterization of the Micrococcus, Staphylococcus, and Streptococcus isolates was completed. Characterization of the micrococci and staphylococci are presented in Table V with their classification. These data indicate that one uncured ham sample from both the hot and cold treatment yielded a possible pathogenic strain of Staphylococcus aureus I (Table V). A subsequent check of the sample code numbers revealed that both cultures were isolated from the same paired hams. This fact would tend to incriminate the animal as the source

TABLE V

BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF GRAM POSITIVE COCCI ISOLATED FROM UNCURED AND CURED HAMS[a]

Identification	No. Isolates	Biochemical Tests							Physiological Characteristics				
		Aerobic					Anaerobic		Hemolysis				
		Fermentation		Nitrate Reduction	Indole	H_2S	Fermentation		Rabbit	Sheep	Pigmentation	Coagulase	Gelatinase
		Glucose	Mannitol				Glucose	Mannitol					
A. Uncured - Hot:													
Staphylococcus aureus I	1	+	+	+	-	-	+	+	α-β	α	G	+	+
Staphylococcus aureus II	3	+	+	+	-	+	+	+	α	α	W	-	+
Staphylococcus epidermidis	4	+	-	+	-	-	+	-	NC	NC	W	-	(+)
Micrococcus luteus	1	+	+	-	-	-	-	-	α[b]	NC	G	-	-
Micrococcus conglomeratus	4	+	-	+	-	-	+	-	NC	NC	Y	-	(+)
Micrococcus candidus	2	+	-	-	-	-	-	-	NC	NC	W	-	-
B. Uncured - Cold:													
Staphylococcus aureus I	1	+	+	+	-	-	+	+	α-β	α	G	+	+
Staphylococcus aureus II	4	+	+	+	-	+	+	+	α	α	W	-	+
Staphylococcus epidermidis	2	(+)	-	+	-	-	+	-	NC	NC	W	-	(+)
Micrococcus conglomeratus	4	+	-	+	-	-	-	-	NC	NC	Y	-	(+)
Micrococcus candidus	4	+	-	-	-	-	-	-	NC	NC	W	-	-
C. Cured - Hot:													
Staphylococcus aureus II	5	+	+	+	-	+	+	+	α	α	W	-	+
Micrococcus candidus	4	+	-	-	-	-	-	-	NC	NC	W	-	-
D. Cured - Cold:													
Staphylococcus aureus II	8	+	+	+	-	+	+	+	α	α	W	-	+
Micrococcus luteus	4	+	+	-	-	-	-	-	α[b]	NC	G	-	-
Micrococcus candidus	2	+	-	-	-	-	-	-	NC	NC	W	-	-

[a] A total of 38 hams analyzed.
[b] Denotes variation from Bergey's Manual description.

+ = Positive for given reaction
- = Negative

G = Golden
W = White

Y = Yellow
NC = No change

α = alpha
α-β = Normal alpha and Beta

( ) = Delayed reaction

of this strain. This strain was compared with the physiological properties of other isolates of Staphylococcus aureus from meat as reported by Jay (1962). No definite comparison could be made to either source or occurrence. Therefore, based upon the tests, the establishment of this isolate as a pathogenic strain of Staphylococcus aureus is only tentative. No public health significance was attached to the remaining 28 staphylococci or micrococci in view of the fact that the source of the isolates was from the uncured tissue. The higher incidence of streptococci in the treatment hams than in the control hams would implicate it as a common inhabitant of freshly slaughtered, porcine tissue. This is in agreement with the finding of Brown et al. (1960). All were positively identified as Streptococcus faecium on the basis of biochemical and physiological characteristics.

The biochemical characterization of the four types of Pseudomonas species isolated is shown in Table VI. No study of flagellation was attempted. The biochemical reactions of this group did not agree sufficiently with those described in Bergey's Manual (Breed et al., 1957) to warrant classification at the species level. Therefore, the four classifications are tentatively designated as Pseudomonas spp. (types I, II, III, and IV) and are included in Table VI. The only organism showing marked proteolytic activity was Pseudomonas species III. The data in Table IV indicate that this organism was the most prevalent type occurring in the uncured tissue of the conventionally processed hams. As a group, however, the majority of the pseudomonads isolated from the hot treatment were characterized as being non-pigmented, did not produce any characteristic odor, and failed to demonstrate any proteolytic activity. Therefore, based upon the characteristics exhibited by the pseudomonads isolated from the uncured tissue from the conventionally processed

TABLE VI

BIOCHEMICAL CHARACTERIZATION OF GRAM NEGATIVE BACTERIA ISOLATED FROM UNCURED AND CURED HAMS[a]

Identification	No. Isolates	Fermentations					Action on Nitrate		Indole	MR-VP	Citrate	Urea	H_2S	Catalase	Oxidase	KCN	Decarboxylase		Ninhydrin	Hydrolysis of	
		Glucose	Lactose	Sucrose	Maltose	Dulcitol	Red	Gas									Arginine	Lysine		Gelatin	Casein
A. Uncured - Hot:																					
Escherichia freundii	5	+	(+)	-	-	+	+	-	-	+/-	+	-	-	+	-	+	-	-	-	(+)	
Pseudomonas sp. I	3	+	-	-	-	-	+	-	-	-/+	+	-	-	+	+		(+)	-		(+)	-
Pseudomonas sp. II	2	+	-	-	-	-	+	-	-	-/-	+	-	-	+	+		±	-		-	-
Pseudomonas sp. III	8	+	-	-	-	-	+	-	-	-/+	-	-	-	+	+		(+)	-		+	(+)
Pseudomonas sp. IV	2	+	+	-	-	-	-	-	-	-/-	+	-	-	+	+		(+)	-		-	-
B. Cured - Hot:																					
Pseudomonas sp. IV	3	+	+	-	-	-	-	-	-	-/-	-	-	-	+	+		(+)	-		-	-
C. Uncured - Cold:																					
Escherichia intermedia	2	+	+	(+)	+	-	+	-	±	-/-	(+)	-	-	+	-	±[b]	-	-	(±)	-	-
Escherichia freundii	3	+	(+)	-	-	+	+	-	-	+/-	+	-	-	+	-	+	-	-	-	(+)	-
Pseudomonas sp. I	2	+	-	-	-	-	+	-	-	-/+	+	-	-	+	+		(+)	-		(+)	-
Pseudomonas sp. II	1	+	-	-	-	-	+	-	-	-/-	+	-	-	+	+		(+)	-		-	-
Pseudomonas sp. III	9	+	-	-	-	-	-	+	-	-/+	-	-	-	+	+		(+)	-		+	(+)
Pseudomonas sp. IV	1	+	+	-	-	-	-	-	-	-/-	+	-	-	+	+		(+)	-		-	-
D. Cured - Cold:																					
Pseudomonas sp. IV	1	+	+	-	-	-	-	-	-	-/-	-	-	-	+	+		(+)	-		-	-

[a] A total of 38 hams analyzed.
[b] Denotes variation from Bergey's Manual description.

+ = Positive for a given reaction
- = Negative

( ) = Delayed reaction
± = Variable

hams, a definite difference is noted. The marked predominance of pseudomonads having a stronger proteolytic tendency agrees with the findings of (Sulzbacher and McLean, 1951; Lepovetsky et al., 1953; Ayres, 1960) for chilled meats.

Bacterial Flora Associated with Cured Tissue

The bacteria isolated from the hot processed cured-cooked samples represented 7 genera. A marked decrease in incidence is indicated in Table IV for all bacteria except Bacillus. The decrease is mainly attributable to exposure of the heat sensitive organisms to the thermal effects, toxic curing ingredients, and low pH. In addition, the rapidity of the application of these combined lethal factors should prevent normal exponential growth of the bacteria. The increase in spore-forming bacilli is attributable to in-process contamination during the boning and curing manipulations for the individual hams. The exhaustive investigations by Smith et al. (1952) showed that thermoduric characteristics for Bacillus species cannot be considered identical due to variation in the maximum and minimum growth temperatures of certain mesophilic strains. Hessen and Riemann (1958) and Riemann (1963) have inferred that bacilli are more common to conventionally processed cured meats than any other genus because of their wide distribution and resistance to thermal processing. The identification of the species occurring in the cured samples from both treatments are shown in Table VII. Photomicrographs of some of the organisms within this group are shown in Figure 3. Comparison of the occurrence of these types in Table VII does not suggest any one specific source of contamination. Based upon the reviews of Smith et al. (1952), Frank (1963), and Riemann (1963) the notable absence of citrate-positive species is apparent for the isolates from both treatments. Otherwise the members of the genus Bacillus

TABLE VII

BIOCHEMICAL CHARACTERIZATION OF SPOREFORMING BACILLI ISOLATED FROM CURED HAMS[a]

Identification	No. Isolates	Acetylmethyl-carbinol	Catalase	Nitrate Reduction	H_2S	Indole	Urease	Citrate	Hydrolysis of			Aerobic Fermentations					Anaerobic Fermentations		
									Casein	Gelatin	Starch	Glucose	Maltose	Sucrose	Mannitol	Glycerol	Glucose	Nitrate	
																		Reduced	Gas
A. Cured - Hot:																			
Bacillus pantothenticus	2	-	+	+	±	-	-	±	+	+	+	+		+	-		(+)	+	-
Bacillus cereus	2	-	+	+	+	-	-	-[b]	+	+	+	+	+	+	+	+	-	+	+
Bacillus laterosporus	2	-	+	+	+	+	-	-	+	+	-	+	-	+	+	-	+	+	-
Bacillus macerans	3	-	+	+	+	-	-	-	-	(+)	+	AG	+	AG	AG	+	AG	+	-
Bacillus polymyxa	1	+	+	+	+	-	-	-	+	+	+	AG	AG	-	AG	AG	AG	+	-
B. Cured - Cold:																			
Bacillus badius	1	-	+	-	-	-	-	-	+	+	(+)[b]	-	-	-	-	(+)	-	-	-
Bacillus cereus	1	-	+	+	+	-	-	-[b]	+	+	+	+	+	+	-	+	-	+	-
Bacillus circulans	1	-	+	+	±	-	-	-	+[b]	+	+	+		+	+	+	+	+	-
Bacillus firmus	3	±	+	+	±	-	-	±	+	+	+	(+)	±	(+)	(+)	-	-	+	-
Bacillus macerans	1	-	+	+	+	-	-	-	-	(+)	+	AG	+	AG	AG	+	AG	+	-

[a] A total of 38 hams analyzed.
[b] Denotes variation from Bergey's Manual description.

AG = Acid and gas formation
± = Variable
+ = Positive for a given reaction
- = Negative
( ) = Delayed reaction

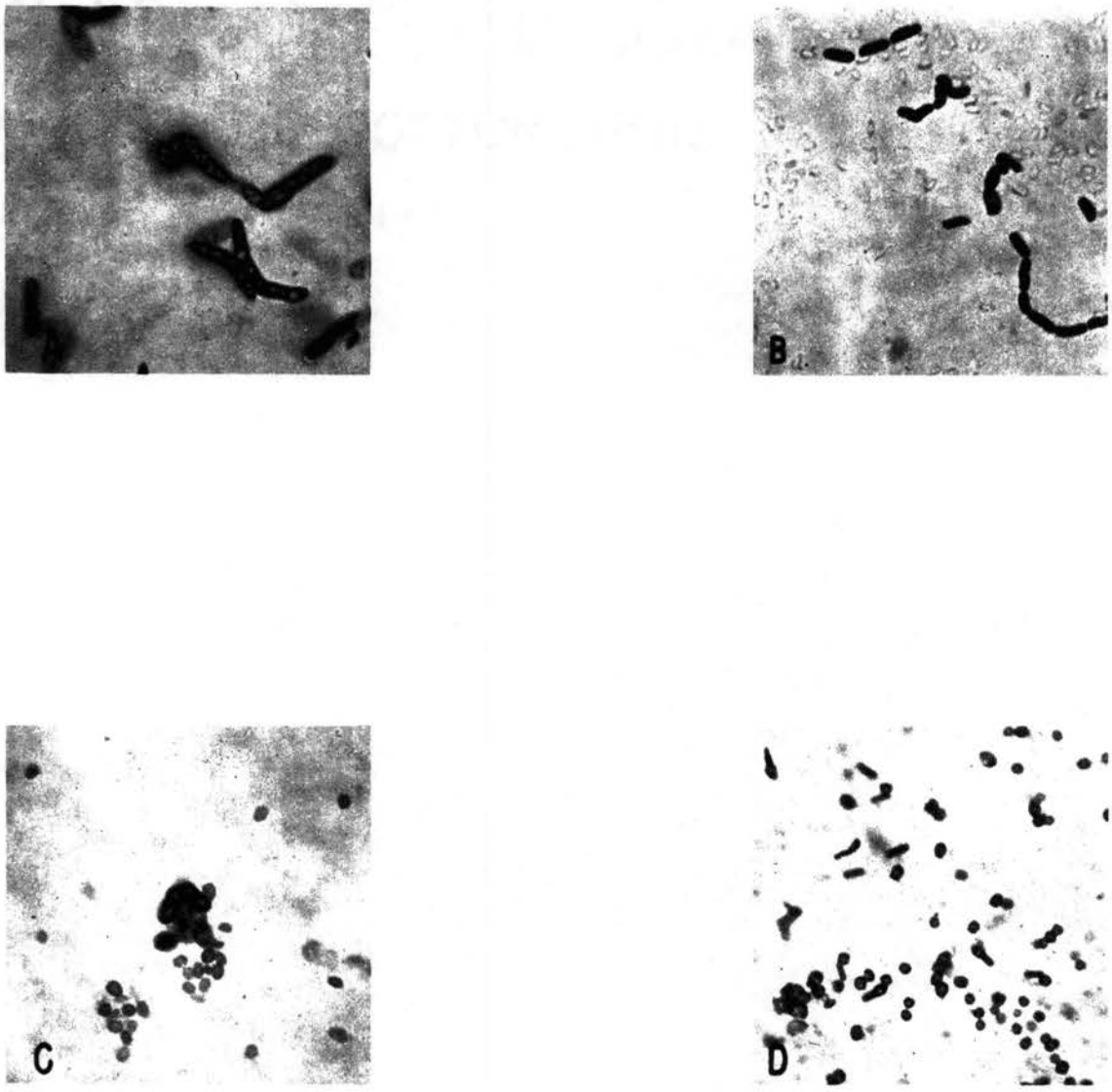


Figure 3. Photomicrographs of aerobic sporeforming bacteria.

A. Bacillus badius, B. B. cereus, C. B. circulans, and D. B. pantothenticus all grown on nutrient agar. Bausch & Lomb "Dynoptic" microscope with 97x oil immersion objective and 10x standard eye-piece as projecting ocular for Leitz Aristophot camera. Koehler critical illumination (1250x; stained by Bartholmew-Mittwer "Cold" technique).

appeared to be about equally distributed in both treatments with no indication that they had any special significance. The characteristics of the two Gram positive rods shown in Table IV did not permit them to be identified further than the genus level. Both cultures exhibited pleomorphism. Because cells from young cultures failed to exhibit the typical snapping division associated with the genus Corynebacterium and no spores were detected, these rods were identified as Bacterium spp. Drake et al. (1960) reported an associative flora from heat processed canned hams containing micrococci and Gram positive rods in which no spores could be detected. They indicated further that the surface count was higher than the count from the inner tissue adjacent to the bone, but that the type of flora was similar in both locations.

In view of the well documented salt tolerance and heat resistance of certain strains of micrococci and staphylococci common to cured meats, little significance was attached to their incidence because none of the 23 colonies tested gave positive coagulase reactions (Dunker et al., 1953; Lechowich et al., 1956).

While the uncured hot processed tissue contained a higher incidence of streptococci, which was reasonable in view of sampling time, they were therefore accepted as natural flora; this incidence was subsequently reduced by processing treatment and the isolates were again confirmed as being Streptococcus faecium. In the uncured cold processed tissue, only one Streptococcus isolate was detected. However, in the cured-cooked tissue an increase was detected (Table IV). The practical significance of these differences is undoubtedly associated with the significant ($P < .05$) treatment effect (Table II). However, within the context of this thesis the precise factor(s) attributable to the decrease can only be conjectured into a combined effect. From a bacteriological approach one can accept the decrease associated with

the hot processed tissue more readily than the cold treatment because of the thermal death characteristics normally displayed by heat tolerant enterococci (Greenberg and Silliker, 1961). However, this same application would not hold true for the enterococci in the same cold processed tissue because of differences in (1) physiological age, (2) transmissibility or acclimazation phenomena, or (3) other factors not yet known. Weiner (1964) postulated that a faster rate of heat removal from pre-rigor injected hams facilitated greater retention of curing solution due to more complete permeation. Conversely, it may be postulated that more thorough heat penetration through the hot processed tissue microstructure effected a greater shock affect upon the enterococci. This coupled with the more thorough permeation of curing ingredients could be another basis for the significant processing effect. In the conventionally processed tissue, rigor is essentially complete when actual processing is commenced 24 hours (or more) post-slaughter. Therefore a "close" tissue microstructure exists (Callow, 1947) and the substrate has become autolyzed. Mossel and Ingram (1955) point out the relationship between alterations in tissue microstructure as effected by completion of rigor mortis and spatial availability of soluble constituents to microorganisms.

The reduction of the associative Gram negative flora is also shown in Table IV for the cured tissue. In view of the limitation previously mentioned regarding the inability to completely identify the members of the genus Pseudomonas, an explanation for the presence of Pseudomonas sp. IV in the cured-cooked samples from both treatments cannot be readily advanced. According to Bergey's Manual, no reported data show any of the members of the genus Pseudomonas to be thermoduric; to the contrary, the majority appear to favor a 25°-30°C temperature

range. Since no proteolytic or lipolytic activity was demonstratable in either isolate, little significance was attached to their low incidence.

Isolation and Classification of Anaerobic Bacteria

The major purpose of this investigation was to ascertain the number and kinds of bacteria on a total count basis that are common to the rapid, hot-processed ham. Only four ham samples yielded anaerobic sporeforming bacteria. Photomicrographs of these four isolates are shown in Figure 4.

Bacterial Flora Associated with Uncured Tissue

The first anaerobe isolated was from the uncured hot processed tissue of the third pig killed. The organism was identified as Clostridium lentoputrescens (Clostridium putrificum). It was characterized as follows: weakly Gram positive, with spherical terminal spores which were not abundant, catalase negative, motile, and delayed reaction with glucose. Hydrogen sulfide was produced in egg-meat medium which was digested after approximately 7 days. Litmus milk was slowly coagulated with ultimate digestion of the casein. Indole production was weakly positive and gelatin was liquified slowly. The anaerobe hemolyzed rabbit and sheep blood agar plates. According to Spray (1936) and Breed et al. (1957) C. lentoputrescens is non-pathogenic.

From the uncured tissue of a conventionally processed ham a culture resembling Clostridium putrefaciens was isolated. However, it is possible that it may be C. tetanomorphum based on maltose fermentation. The main characteristics of this isolate are given as follows: Gram positive with spherical terminal to sub-terminal spores which swell the cell only slightly. According to McBryde (1911) the bacillus

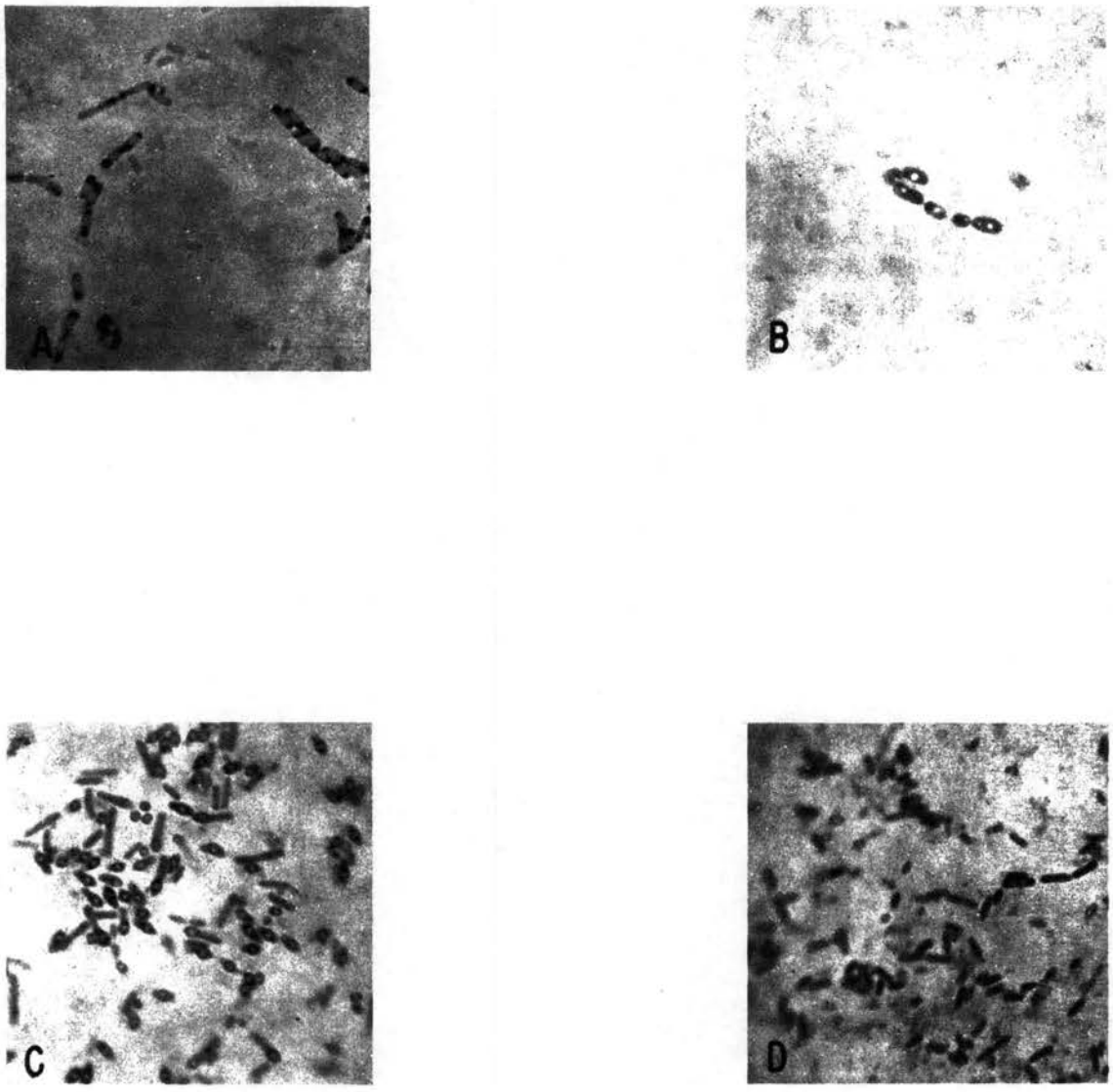


Figure 4. Photomicrographs of anaerobic sporeforming bacteria.

A. Clostridium bifermentans, B. C. lentoputrescens, C. C. putrefaciens, and D. C. sporogenes all grown on brain-heart agar. Bausch & Lomb "Dynoptic" microscope with 97x oil immersion objective and 10x standard eyepiece as projecting ocular for Leitz Aristophot camera. Koehler critical illumination (1250x; stained by Bartholmew-Mittwer "Cold" technique).

possess no motility. The organism produced hydrogen sulfide in small patches and was found to be negative for both nitrite and indole. The organism was inconsistent in producing gas from glucose. Delayed acid with slight gas production sometimes occurred in both glucose and maltose after 15 days. Bacto egg-meat medium was disintegrated and slowly digested. Poor growth at 37°C was exhibited and optimum growth appeared to be at normal room temperature. The organism hemolyzed rabbit blood agar but appeared to have a weak hemolysis pattern on sheep blood. It is described in Bergey's Manual to be non-pathogenic (Breed et al., 1957).

The selective effect of the holding environment on the germination of exogenous spores was indicated in the case of C. putrefaciens.

Bacterial Flora Associated with Cured Tissue

The first anaerobe isolated from the cured-cooked samples was from a hot processed ham and identified as Clostridium bifermentens. Its diagnostic features are: strict anaerobe, catalase negative, motile, non-swollen rods with eccentric ovoid to cylindrical spores. Iron milk was digested and blackened in 24-30 hours. The organism was nitrite negative, hydrogen sulfide and indole positive and liquefied gelatin. Glucose, sucrose, and salicin were fermented. Precipitation was produced on egg yolk agar without definite luster. Tyrosine crystals were formed within 10 days in Bacto egg-meat medium. The organism was described by Spray (1936) as non-pathogenic. The last anaerobe isolated was from a conventionally processed ready-to-eat ham. This isolate was readily identified as Clostridium sporogenes by its litmus milk reaction. Tyrosine crystals were observed in Bacto egg-meat medium after 15 days. Both rabbit and sheep blood were hemolyzed. According to Bergey's Manual, this anaerobe is non-pathogenic for humans (Breed et al., 1957).

SUMMARY AND CONCLUSIONS

The bacterial populations studied in the rapid, hot processed ham revealed no significant bacteriological problems in the new concept of complete processing prior to chilling. Quantitatively, both uncured and cured tissues from 38 treatment and control pairs were enumerated on a total count basis. The incidence of aerobic bacterial flora in the rapid processed ham is comparable to that in the conventional processed ham. However, greater reductions in total bacterial numbers were obtained with rapid processing techniques. From a bacteriological standpoint, the combined effects of pH, curing ingredients, smoking, cooking, and rapidity of handling the product facilitate the exposure of the associative bacteria to maximum lethal factors, thus effecting a significantly ($P<.05$) greater reduction in the bacterial load of the ready-to-eat ham.

The qualitative aspects of the rapid cured ready-to-eat ham likewise appear to be quite comparable to the conventionally processed product. However, in view of the reduced incidence of various enterococci from the finished product, it would appear that the hot processed ham would be more stable. Since the spoilage of a cured meat product depends to a large extent upon the level and types of initial viable bacterial numbers and their resistance to treatment, competition, and ability to grow under the conditions of storage, the rapid cured ready-to-eat ham appears to present advantages over the conventional method of ham curing. The bacterial flora of the rapid cured, pre-rigor ham was primarily composed of Gram positive

sporeforming bacilli, staphylococci, and micrococci, most of which were facultative anaerobes having varying degrees of proteolytic activity. All of the clostridia isolated were of the putrefactive type. They appeared to be intermediate between C. sporogenes and C. bifermentans. None of the samples from either treatment contained pathogenic bacteria in the ready-to-eat product.

A comparison of the final mean pH values and residual moisture indicate that rapid hot processing does not appreciably affect the ultimate pH or moisture retainability of the product. Therefore, economic as well as public health benefits would be expected if the process is adopted and adequate control is effected. Regardless of industrial adoption, the closer the time of processing completion is to the time of slaughter, the less opportunity exists for undesirable bacterial growth to occur. However, it appears that more definitive studies of the rapid cured, pre-rigor ham should be accomplished with known quantities of applicable heat tolerant test bacteria prior to drawing further conclusions regarding specific ecological or stability attributes of the product.

Conclusions from this investigation reveal that by eliminating the initial chill treatment one can combine the lethal factors associated with processing in a manner in which undesirable bacteria, from both the standpoint of numbers and types, can be significantly reduced. By application of these methods utilizing the recommendations of the Meat Inspection Division, U.S.D.A. as guidelines for hygienic and sanitation control, a bacteriologically sound product is possible.

LITERATURE CITED

American Meat Institute Foundation. 1960. The Science of Meat and Meat Products. W. H. Freeman and Co., Inc., San Francisco.

American Public Health Association. 1953. Standard Methods for the Examination of Dairy Products. 10th ed. Amer. Public Health Assn., Inc. New York.

_____. 1960. Standard Methods for the Examination of Water and Wastewater. 11th ed. Amer. Public Health Assn., Inc. New York.

Angelotti, R., M. J. Foter, and K. H. Lewis. 1961. Time-temperature effects on salmonellae and staphylococci in foods. III. Thermal death time studies. Appl. Microbiol. 9:308-315.

_____, G. C. Bailey, M. J. Foter, and K. H. Lewis. 1961. Salmonella infantis isolated from ham in food poisoning incident. Public Health Rep. 76:771-776.

Appleman, M. D. 1957. Symposium on problems in the microbiological analysis of foods. Bacteriol. Rev. 21:241-245.

Association of Official Agricultural Chemists. 1960. Official Methods of Analysis. 9th ed., Assn. of Off. Agri. Chemists. Washington, D. C.

Ayres, J. C. 1955. Microbiological implications in the handling, slaughtering and dressing of meat animals. Advanc. Food Res. 6:109-161.

_____. 1960. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. Food Res. 25(1):1-18.

Baltzer, J. and D. C. Wilson. 1965. The occurrence of clostridia on bacon slaughter lines. J. Appl. Bact. 28(1):119-124.

Batcher, O. M., G. L. Gilpin, N. R. Duckworth, and P. W. Finkel. 1964. Eating quality of quick-cured hams. J. Home Econ. 56(10):758-762.

Bate-Smith, E. C. 1948. The physiology and chemistry of rigor mortis. Advanc. Food Res. 1:1-38.

Bendall, J. R., O. Hallund, and J. Wismer-Pedersen. 1963. Post-mortem changes in the muscles of Landrace pigs. J. Food Sci. 28(2):157.

Berman, M. D. and C. E. Swift. 1964. Meat curing: The action of NaCl on meat electrolyte binding. J. Food Sci. 29(2):182-189.

Bratzler, L. J. 1958. Fifty years of progress in meat research. J. An. Sci. 17(4):1079-1088.

Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey's Manual of Determinative Bacteriology, 7th ed., Williams and Wilkins Co., Baltimore, Md.

Briskey, E. J. and J. Wismer-Pedersen. 1961. Biochemistry of pork muscle structure. I. Rate of anaerobic glycolysis and temperature change versus the apparent structure of muscle tissue. J. Food Sci. 26(3):297-304.

Briskey, E. J., R. N. Sayre, and R. G. Cassens. 1962. Development and application of an apparatus for continuous measurement of muscle extensibility and elasticity before and during rigor mortis. J. Food Sci. 27(6): 560-566.

Brissey, G. E. 1952. U. S. Patent. Preparing cooked cured meats. U. S. Government Patent Office, Washington, D. C.

Brown, W. L., C. A. Vinton, and C. E. Gross. 1960. Heat resistance and growth characteristics of microorganisms isolated from semi-perishable canned hams. Food Res. 25(3):345-349.

Burke, M. V., K. H. Steinkraus, and J. C. Ayres. 1950. Methods for determining the incidence of putrefactive anaerobic spores in meat products. Food Technol. 4:21-25.

Callow, E. H. 1947. The action of salts and other substances used in the curing of bacon and ham. Brit. J. Nutrit. 1:269-274.

Camp, B. J. and C. Vanderzant. 1957. Proteolytic enzymes from Pseudomonas putrefaciens. II. Characteristics of an endocellular proteolytic enzyme system. Food Res. 22:158.

Casman, E. P., D. W. McCoy, and P. J. Brandley. 1963. Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 11:498-500.

Cochran, W. G. 1950. Estimation of bacterial densities by means of the "most probable number." Biometrics 6:105-116.

Cosnett, L. S., D. J. Hogan, N. H. Law, and B. B. Marsh. 1956. Bone-taint in beef. J. Sci. Food Agri. 7:546-551.

Curran, H. R. and M. J. Pallansch. 1963. Incipient germination in heavy suspensions of spores of Bacillus stearothermophilus at subminimal growth temperatures. J. Bacteriol. 86:911-916.

Dack, G. M. 1964. Characteristics of botulism outbreaks in the United States. In Proc. of a symposium on botulism; Lewis and Cassel, eds., Cincinnati, Ohio, pp. 33-40.

Dauer, C. C. 1961. 1960 summary of disease outbreaks and a 10-year résumé. Public Health Rep. 76(10):915-922.

Deatherage, F. E. and R. Hamm. 1960. Influence of freezing and thawing on hydration and charges of the muscle proteins. Food Res. 25(5):623.

Deibel, R. H. and C. F. Niven, Jr. 1958. Microbiology of meat curing. I. The occurrence and significance of a motile microorganism of the genus Lactobacillus in ham curing brines. Appl. Microbiol. 6:323-326.

Difco Laboratories, Inc. 1953. Difco Manual, 9th ed., Difco Laboratories, Detroit, Mich.

Drake, S. D., J. B. Evans, and C. F. Niven, Jr. 1960. The effect of heat and irradiation on the microflora of canned hams. Food Res. 25(2):270-278.

Draudt, H. N. 1963. The meat smoking process: A review. Food Technol. 17:1557-1598.

Dunker, C. F., M. Berman, G. G. Snider, and H. S. Tubiash. 1953. Quality and nutritive properties of cured hams. III. Vitamin content, biological value of protein and bacteriology. Food Technol. 7:288-290.

Edelmann, R., J. R. Mohler, and A.Eichhorn. 1945. Meat Hygiene. 8th ed., Lea & Febiger, Philadelphia.

Elliott, R. P. and H. D. Michener. 1965. Factors affecting the growth of psychrophilic microorganisms in foods: A review. U.S.D.A., ARS. Tech. Bul. No. 1320, pp. 1-110.

Frank, H. A. 1963. Factors affecting bacterial spoilage of animal products at elevated temperatures. Food Technol. 17(5):573-578.

Frazier, W. C. 1958. Food Microbiology. McGraw-Hill Book Co., New York.

Galton, M. M., W. V. Smith, H. B. McElrath, and A. B. Hardy. 1954. Salmonella in swine, cattle and environment of abattoirs. J. Infect. Dis. 95:236-245.

Gibbs, B. M. and B. Freame. 1965. Methods for the recovery of clostridia from foods. J. Appl. Bact. 28(1):95-116.

Graves, R. R. and W. C. Frazier. 1963. Food microorganisms influencing the growth of Staphylococcus aureus. Appl. Microbiol. 11:513-516.

Greenberg, R. A. and J. H. Silliker. 1961. Evidence for heat injury in enterococci. J. Food Sci. 26:622-625.

Greenberg, R. A. 1965. Personal Communication.

Hall, J. L., D. L. Harrison, B. D. Westerman, L. L. Anderson, and D. L. Mackintosh. 1961. Effects of preslaughter feeding and resting of transported swine on quality of pork products. Kansas Agr. Exp. Sta. Tech. Bul. No. 119.

Hall, H. E. and R. Angelotti. 1965. Clostridium perfringens in meat and meat products. Appl. Microbiol. 13:352-357.

Hamm, R. 1959. Biochemistry of meat hydration. In Proc. of the 11th Res. Conf., A.M.I.F., Cit. No. 50, p. 17.

Hamm, R. 1960. Biochemistry of meat hydration. Advanc. Food Res. 10:356.

Hansen, R., R. Rogers, S. Emge, and N. J. Jacobs. 1964. Incidence of Salmonella in the hog colon as affected by handling practices prior to slaughter. J. Amer. Vet. Med. Assn. 145(2):139-140.

Heather, C. D. and C. Vanderzant. 1957. Effects of temperature and time of incubating and pH of plating medium on enumerating heat-treated psychrophilic bacteria. J. Dairy Sci. 40(9):1079-1086.

Heck, M. C., E. S. Ruby, and M. J. Burris. 1955. Factors affecting the preservation of cured pork. Arkansas Agri. Exp. Sta. Bul. No. 559.

Henrickson, R. L., I. T. Omtvedt, D. Hammons, and R. W. Mandigo. 1965. The influence of "hot-processing" on the quality of pork products. Cooperative Agreement 12-25-020-2456. Okla. State Univ., Stillwater, Okla.

Hessen, I. and H. Riemann. 1958. Sporeforming bacteria in canned meats. The Royal Vet. Agr. Col. Ann. Yearbook. pp. 50-68. Copenhagen.

Hobbs, B. C. 1965. Clostridium welchii as a food poisoning organism. J. Appl. Bact. 28(1):74-82.

Ingram, M. 1952. Internal bacterial taints ('Bone taint' or 'souring') of cured pork legs. J. Hyg. 50:165-179.

Jay, J. M. 1962. Further studies on staphylococci in meat. III. Occurrence and characteristics of coagulase-positive strains from a variety of nonfrozen market cuts. Appl. Microbiol. 10:247-251.

_____. 1965. Relationship between water-holding capacity of meats and microbial quality. Appl. Microbiol. 13:120-121.

Jensen, L. B. and W. R. Hess. 1941. A study of ham souring. Food Res. 6:273-308.

Jones, D., R. H. Deibel, and C. F. Niven, Jr. 1963. Identity of Staphylococcus epidermidis. J. Bacteriol. 85(1):62-67.

Kamm, L., J. C. Bartlet, and D. M. Smith. 1964. Assessing gain in the lean of hams. Food Technol. 18(10):144-148.

Kastenschmidt, L. L., E. J. Briskey, and W. G. Hoekstra. 1964. Prevention of pale, soft, exudative porcine muscle through regulation of ante-mortem environmental temperature. J. Food Sci. 29(2):210-217.

Lechowich, R. V., J. B. Evans, and C. F. Niven, Jr. 1956. Effect of curing ingredients and procedures on the survival and growth of staphylococci in and on cured meats. Appl. Microbiol. 4(6):360-363.

Lepovetsky, B. C., H. H. Weiser, and F. E. Deatherage. 1953. A microbiological study of lymph nodes, bone marrow and muscle tissue obtained from slaughtered cattle. Appl. Microbiol. 1:57-59.

Levie, A. 1963. The Meat Handbook. AVI Pub. Co., Westport, Conn.

Lewis, K. H. and R. Angelotti. 1964. Examination of Foods for Enteropathogenic and Indicator Bacteria. Public Health Serv. Pub. No. 1142, pp. 1-123.

Lowe, B. 1955. Experimental Cookery. 4th ed. John Wiley & Sons, Inc. New York. Chapter 8.

Mandigo, R. W. and R. L. Henrickson. 1965a. Influence of hot processing pork carcasses on cured ham. Food Technol. (In print).

_____ and R. L. Henrickson. 1965b. Influence of hot processing techniques on various bacon parameters. Food Technol. (In print).

McBryde, C. N. 1911. A bacteriological study of ham souring. U.S.D.A. Bur. Anim. Ind. Bul. No. 132., pp. 1-55.

McClung, L. S. and R. Toabe. 1947. The egg yolk plate reaction for the presumptive diagnosis of Clostridium sporogenes and certain species of the gangrene and botulinum groups. J. Bacteriol. 53:139-147.

McLean, R. A. and W. L. Sulzbacher. 1959. Production of flavor in cured meat by a bacterium. Appl. Microbiol. 7:81-83.

Merchant, I. A. and R. A. Packer. 1961. Veterinary Bacteriology and Virology. 6th ed., Iowa State Univ. Press, Ames.

Miller, A. R. U.S.D.A., Meat Inspection Branch Memo. No's 205 (July 1, 1954), 217 (June 15, 1955) and 218 (April 14, 1955).

Mossel, D. A. A. and M. Ingram. 1955. The physiology of the microbial spoilage of foods. J. Appl. Bacteriol. 18:232-264.

_____ and H. J. L. Van Kuijk. 1955. A new and simple technique for the direct determination of equilibrium relative humidity of foods. Food Res. 20:415-423.

Mullins, A. M., G. G. Kelley, and D. E. Brady. 1958. The effect of various additives on the stability of cured hams. Food Technol. 12(5):227-230.

Mundt, J. O., C. J. Mayhew, and G. Stewart. 1954. Germination of spores in meats during cure. Food Technol. 8:435-436.

Niven, C. F., Jr. 1960. Some prejudices and outmoded beliefs in meat curing. A.M.I.F. Cir. No. 56, pp. 1-17.

_____. 1961. Microbiology of Meats. A.M.I.F. Cir. No. 68,pp.1-7.

_____. 1964. Meat industry's responsiblities. In Proc. of Nat. Conf. on Salmonellosis. Atlanta, Ga. pp. 155-157.

Parker, S. 1952. Preservation of smoked meats. Refrig. Enr. 60(11):1177-1178.

Partmann, W. 1963. Postmortem changes in chilled and frozen muscle. J. Food Sci. 28(1):15-27.

Pearson, A. M., W. D. Baten, A. J. Goembel, and M. E. Spooner. 1962. Application of surface-response methodology to predicting optimum levels of salt and sugar in cured ham. Food Technol. 16(5):137-138.

Peterson, A. C., J. J. Black, and M. F. Gunderson. 1964. Staphylococci in competition. III. Influence of pH and salt on staphylococcal growth in mixed populations. Appl. Microbiol. 12(1):70-76.

Pivnick, H. and H. Bird. 1965. Toxinogenesis by Clostridium botulinum types A and E in vacuum-packed prepared meats. Food Technol. 19(7):132-140.

_____ and H. Barnett. 1965. Effect of salt and temperature on toxinogenesis by Clostridium botulinum in perishable cooked meats vacuum-packed in air-impermeable plastic pouches. Food Technol. 19(7):140-143.

Porter, J. E. 1965. The Salmonella problem. In Proc. Md. Nutr. Conf., Washington, D.C., pp. 1-8.

Pulliam, J. D. and D. C. Kelley. 1965. Bacteriological comparison of hot processed and normally processed hams. J. Milk and Food Technol. 28: 285-286.

Reith, A. F. 1926. Bacteria in the muscular tissues and blood of apparently normal animals. J. Bacteriol. 12:367-381.

Riemann, H. 1963. Safe heat processing of canned cured meats with regard to bacterial spores. Food Technol. 17(1):39-49.

Rose, D. and R. Peterson. 1953. Non-bacterial reduction of nitrite in pork. Food Technol. 7(9):369-372.

Rubin, H. L., M. Scherago, and R. H. Weaver. 1942. The occurrence of Salmonella in the lymph glands of normal hogs. Amer. J. Hyg. 36:43-47.

Saffle, R. L. and J. W. Cole. 1960. Fasting effects on dressed yields, shrink and pH of contractile tissue in swine. J. Animal Sci. 19:242.

Shank, J. L., J. H. Silliker, and R. H. Harper. 1962. The effect of nitric oxide on bacteria. Appl. Microbiol. 10:185-189.

Silliker, J. H. 1959. The effects of curing salts on bacterial spores. In Proc. of the 11th Res. Conf. A.M.I.F. Cir. No. 50, p. 51.

Silliker, J. H., C. E. Jansen, M. M. Voegell, and N. W. Chmura. 1962. Studies on the fate of staphylococci during the processing of hams. J. Food Sci. 27:50-56.

Smith, N. R., R. E. Gordon, and F. E. Clark. 1952. Aerobic Sporeforming Bacteria. U.S.D.A. Monogr. No. 16, pp. 1-148.

Smith, R. E., G. L. Nelson, and R. L. Henrickson. 1965. Analyses on transient heat transfer from anomalous shapes. Paper No. 65-806, Amer. Soc. Agri. Engr., Chicago.

Snedecor, G. W. 1956. Statistical Methods. 5th ed. The Iowa State College Press, Ames.

Society of American Bacteriologists. 1957. Manual of Microbiological Methods. McGraw-Hill Book Co., Inc., New York.

Spray, R. S. 1936. Semisolid media for cultivation and identification of the sporulating anaerobes. J. Bacteriol. 32:135-154.

Steinkraus, K. H. and J. C. Ayres. 1964. Incidence of putrefactive anaerobic spores in meat. J. Food Sci. 29(1):87-93.

Strong, D. H., J. C. Canada, and B. B. Griffiths. 1963. Incidence of Clostridium perfringens in American foods. Appl. Microbiol. 11:42-44.

Sulzbacher, W. L. and R. A. McLean. 1951. The bacterial flora of fresh pork sausage. Food Technol. 5(1):7-8.

Sulzbacher, W. L. 1953. Report of subcommittee on microbiological methods of meat research. In Proc. 5th Recip. Meat Conf., p. 170.

Sulzbacher, W. L. 1957. Associated bacteriological and chemical changes in meat curing brines. In Proc. of Second Intern. Symp. on Food Microbiol. Cambridge, England, pp. 315-323.

United States Department of Agriculture: 1959. Manual of Inspection and Sanitation Requirements of the Federal Meat Grading Service. U.S.D.A., AMS, Washington, D.C.

United States Department of Agriculture: 1962. Manual of Meat Inspection Procedures of the United States Department of Agriculture, Revised, U.S.D.A., AMS, Washington, D.C.

United States Department of Agriculture. 1964. Manual of Meat Inspection Procedures of the United States Department of Agriculture, CFR Amend. 64-61, pp. 1-7.

United States Public Health Service. 1962. Morbidity and Mortility Weekly Rep. 10(3):247.

Upadhyay, J. and J. L. Stokes. 1962. Anaerobic growth of psychrophilic bacteria. J. Bacteriol. 83:270-275.

Vanderzant, C. 1957. Proteolytic enzymes from Pseudomonas putrefaciens. I. Characteristics of an extracellular proteolytic enzyme system. Food Res. 22:151-154.

Weiner, P. D. 1964. The effect of processing pork carcasses prior to rigor mortis upon muscle and fat quality. M. S. Thesis, Kansas State Univ.

Wismer-Pedersen, J. 1960. Effect of cure on pork watery structure, binding of salt and water to the meat. Food Res. 25:789.

Wistreich, H. E., R. E. Morse, and L. J. Kenyon. 1959. Curing of ham: A study of sodium chloride accumulation. I. Methods, effects of temperature, cations, muscles and solution concentration. Food Technol. 13(8):441-443.

_____. 1960. Curing of ham: A study of sodium chloride accumulation. II. Combined effects of time, solution concentration and solution volume. Food Technol. 14(11):549-551.

Wodzinski, R. J. and W. C. Frazier. 1961. Moisture requirements of bacteria. V. Influence of temperature and decreased partial pressure of oxygen on requirements of three species of bacteria. J. Bacteriol. 81:409-415.

Zatovil, O. 1963. Nitrite effect. Fleischwirtschaft. 15:798.

APPENDICES

APPENDIX A

METHODS FOR PREPARING SPECIAL BACTERIOLOGICAL MEDIA

I. Preparation of blood agar plates

Materials:

Bacto Tryptose Blood Agar Base
Defibrinated blood[a]

1. Rehydrate basal medium following manufacturer's instructions.
2. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121°C).
3. Allow sterile base to cool to 45°C and while still liquid, add 4 percent sterile blood. Agitate thoroughly, and dispense aseptically into sterile 100 mm. petri dishes. Any air bubbles can be removed immediately by momentarily raising lid and flaming the surface with a bunsen burner.
4. Allow to solidify and then invert plates and incubate at 37°C for 24 hours to check sterility.

II. Preparation of egg yolk agar plates

Materials:

Fresh, shell eggs
Proteose peptone
Glucose
Agar
$MgSO_4$
NaCl
$Na_2HPO_4 \cdot 7HOH$
KH_2PO_4

1. Strict aseptic procedures are necessary for separating the yolk from the egg white. After scrubbing the shells with detergent, immerse whole eggs individually in an ethyl alcohol bath. Flame while saturated and break shell with a sterile knife and then separate yolk by routine culinary technique. Aseptically place required number of yolks in a sterile graduate cylinder. Add an equal volume of sterile saline, mix thoroughly. Maintain at room temperature.

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[a] Sterile defibrinated sheep and rabbit blood was obtained from Colorado Serum Company, Denver, Colorado.

APPENDIX A (Continued)

2. Prepare basal medium following the McClung-Toabe formula (McClung and Toabe, 1947) and sterilize in the autoclave for 15 minutes at 15 pounds pressure (121°C).
3. Allow sterile base to cool to 50°C and while still liquid, add 10 ml of the sterile egg yolk suspension. Mix thoroughly and dispense aseptically into sterile 100 mm petri dishes. Remove any air bubbles.
4. Check sterility prior to use.

III. Preparation of fortified TGEA plates

Materials:

Bacto tryptose glucose beef extract agar
$CaCl_2$
$MnSO_4$

1. Basal medium was rehydrated using deionized-distilled water to which 0.05 grams $MnSO_4$ and 0.1 gram of $CaCl_2$ per liter were added.
2. Sterilize in the autoclave for 15 minutes at 15 pounds pressure (121°C).
3. Dispense sterile media into 100 mm petri dishes.
4. Check sterility prior to use.

IV. Preparation of NBSFA plates

Materials:

1% aqueous nile blue sulfate solution
Purified corn oil
Bacto plate count agar

1. The NBSFA medium was prepared using a modification of the medium described by Sulzbacher (1951). The medium prepared consisted of 500 ml of plate count agar to which 5 ml of a freshly filtered 0.1 percent aqueous solution of nile blue sulfate solution and 5 ml of corn oil was added.
2. The fat emulsion-mixture was sterilized in the autoclave and while still hot was mixed at the low speed setting for 1 minute in a sterile Waring blendor jar. The plates were poured directly from the blendor jar and when solidified were incubated at 37°C to assure sterility.

APPENDIX B

Total Aerobic Mesophilic Bacterial Counts[a]

Sample No.	Hot		Cold	
	Uncured	Cured	Uncured	Cured
1	7	31	230	12
2	618	12	180	25
3	6	9	9	53
4	27	3	28	1
5	90	109	1	<1
6	190	<1	441	1
7	TNTC[b]	—[c]	TNTC	—
8	184	1	618	<1
9	2191	7	1352	27
10	6	<1	3	2
11	5	4	12	36
12	144	65	1	4
13	77	1	8	<1
14	181	1	128	305
15	9	1	18	15
16	30	12	507	1327
17	56	3	10	69
18	25	15	366	97
19	23	16	220	2
20	5	2	<1	72

[a] Bacteria per gram of original sample.
[b] TNTC - To numerous to count (range estimated at greater than 3×10^6).
[c] Not counted.

APPENDIX C

Total Anaerobic Bacterial Counts

Type	MPN SERIES 10	1.0	0.1	Tabular MPN Value[a]	Anaerobes/gm.
Uncured (Hot)	1	0	0	2	<1
Uncured (Cold)	1	0	0	2	<1
Cured (Hot)	2	0	0	4.5	4
Cured (Cold)	1	0	0	2	<1

[a] Adapted from "Standard Methods for the Examination of Water and Sewage", 11th ed.

VITA

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