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A large number of bacterial and yeast isolates were obtained from meat and poultry processing plants by swab and contact plate sampling methods. Most of the isolates were subjected to a brief taxonomic study and listed according to their sources. Representative food spoilage, indicator and pathogenic organisms were identified to genus and species, and germicide effectiveness studies were performed on them.

The organisms tested were Escherichia coli, Pseudomonas aeruginosa, Streptococcus faecalis, Micrococcus luteus, Salmonella derby, spores of Bacillus licheniformis, and yeast of the genus Candida. The method of preparing cultures and evaluating germicides was that of Chambers. Each isolate was exposed to varying concentrations of four different germicides in both soft (distilled) and hard

(USDA - 500 ppm $CaCO_3$) water, and the bactericidal effectiveness of each was measured at time intervals varying from 15 to 300 seconds. The four germicides tested were sodium hypochlorite, a moderately acidic iodophor, a highly acidic iodophor and a quaternary ammonium compound (QAC).

Results of the germicide studies reflect the similar effectiveness of hypochlorite and iodophor compounds and the superiority of both of these types of germicides over the QAC at any given concentration.

The yeasts, however, were considerably more resistant to the hypochlorite than to the iodophors. This probably was due to the relatively higher concentration of organic matter in the yeast cells as opposed to bacterial cells, and reflected the greater susceptibility of chlorine to the presence of organic matter in the germicide solution. Also, iodophors have been reported to be highly active against yeast alcohol and glucose-6-phosphate dehydrogenases.

Spore suspensions were more susceptible to hypochlorite than to the iodophors, and a marked increase in sporicidal activity was noted when hypochlorite solutions were buffered down to pH 7.1.

Germicide Effectiveness and Taxonomic Studies on Microbial Isolates from Meat and Poultry Plants

by

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This thesis is dedicated to my wife, Mari.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GERMICIDE EFFECTIVENESS AND TAXONOMIC STUDIES ON MICROBIAL ISOLATES FROM MEAT AND POULTRY PLANTS

INTRODUCTION

Increasing governmental and consumer concern for food safety and purity has led food processors to review their in-plant sanitation procedures and to seek more effective methods of minimizing product contamination.

Advances made in this area by the dairy industry have exemplified the effectiveness of such programs. Germicides have played an important role in dairy sanitation, and many of their applications can be readily adapted to other types of food processing.

Most dairy processing equipment is enclosed within pipes and vats, while such protection is not available to all forms of food processing. Meat and poultry handling equipment necessitate large surface areas which are repeatedly exposed to aerosol, waterborne and product contact contamination. Transfer and build up on these surfaces represent a significant problem to processors both in terms of reduced shelf life and transmission of pathogenic organisms to the consumer.

Most fresh beef, pork and poultry meat is processed without the use of final product decontamination prior to reaching the market. Increasingly widespread use of prepacking procedures have further

focused attention on the need for rigorous sanitation at the processing plant, which must be considered the primary source of contamination.

This study deals with the comparative effectiveness of various types of germicides in the destruction of bacterial, yeast and spore contamination obtained from meat and meat processing surfaces. Both soft and hard water were used as germicide diluents to represent the extremes found in processing plant conditions.

A typical spectrum of representative spoilage, indicator and pathogenic isolates was selected for use in the germicide tests. The selected isolates were exposed to different germicides for periods of 15, 30, 60, 120 and 300 seconds under varying conditions of germicide concentration and water hardness. Bacterial spores underwent exposures lasting as long as 20 minutes.

Properties of various germicides are discussed, and their relative efficiency against the common microflora of meat and poultry are compared.

REVIEW OF LITERATURE

Contamination of Meat and Poultry

Source of Contamination

Organisms from the soil, air, water, and waste materials contribute to the microbial load on the external surfaces of animals. These organisms are transmitted to the meat by contact with the workers and equipment associated with the slaughtering and processing operation (100, p. 176).

Many factors affect the level and type of contamination entering meat processing plants. Grau, Brownlie and Roberts (34) reported that the percentage of cattle carrying Salmonella depended upon the pre-slaughter treatment of the animals. Prolonged holding and feeding between the farm and slaughter exposes cattle to contamination common in transport vehicles, holding pens, and abattoirs. Jensen (50, p. 165-168) indicated that the physiological condition of ante-mortem beef greatly affects the nature and degree of post-processing spoilage. The extent of in-plant cross contamination of the meat is also affected since spoilage begins before the meat leaves the processing plant.

Sauter, Ardrey and Petersen (77) noted a marked reduction in shelf life of chickens processed less than two weeks after infection

with Escherichia coli, but that the number of E. coli decreased during refrigerated holding. The authors concluded that the spoilage organisms (predominantly pseudomads) were able to grow more rapidly on fryers that were infected with E. coli shortly before processing.

Hamdy, Barton and Brown (39) reported that bruised tissues in poultry were highly susceptible to prolonged survival of Staphylococcus aureus. Thus, in addition to contamination from skin, feathers, hides, feet and excreta, the tissues of the meat itself may provide the vehicles for entry of pathogenic contaminants into the processing plant environment.

In-Plant Contamination

A study by Gunderson, McFadden and Kyle (36) described the bacteriology of commercial poultry processing and detailed the bacterial populations associated with each phase of processing. They concluded that a decrease in skin contamination occurred during the evisceration process, probably due to vigorous washing of the carcasses in the final stages. Holding several eviscerated carcasses in common receptacles, such as chill tanks, was found to increase and distribute contamination on all the carcasses. Such storage is common practice in poultry processing.

The sanitation of individual plants plays an important role in determining final counts. Surkiewicz, et al. (86) examined nine

chicken evisceration plants and found that the great majority of eviscerated chickens yielded aerobic plate counts of 25,000 per gram (15,000 or fewer per cm^2 of skin surface). Walker and Ayres (94) in a similar study reported that the skin of live birds had counts of approximately 1,500 organisms per cm^2, while the skin of eviscerated carcasses yielded counts of approximately 35,000 per cm^2. They concluded that the processing line was an important avenue for disseminating microorganisms.

Most poultry processing plants utilize some form of conveyor system by which the carcasses are hung and moved from the killing area, through the cleaning and evisceration lines, and on to the storage or chilling area, a period of exposure which varies up to an hour, depending upon the size and speed of the operation. Airborne microorganisms were sampled in a study of a broiler processing plant by Kotula and Kinner (53). They reported that dressing room air counts reached 7,000 per ft^3 during operation of the plant.

Processing plant workers may also contribute to the pool of contamination within the plant environment. Hall and Hauser (38) examined 219 fecal specimens from food handlers in Louisiana and found 78 percent contaminated with Clostridium perfringens, and 79.9 percent contaminated with enteropathogenic E. coli, although no Salmonella or Shigella were isolated from the individuals tested. Contamination from nasal, suppurative, expectorative and fecal

sources represent a significant hazard in plants where conditions of sanitation and worker hygiene are not vigorously pursued (88, p. 33).

Mesophilic Pathogenic and Spoilage Organisms

Ingram and Dainty (46) reported that the microflora of meat and the spoilage they cause is a function of temperature. Under warm conditions the predominant species are clostridia, which grow in meat under reducing conditions (e.g., within the muscle) so rapidly that they precede spoilage at the surface. Clostridia are more frequently being cited as the cause of outbreaks of gastroenteritis. Bryan (12) reported surveys of market foods which indicated a high incidence of C. perfringens: raw meats, 58 percent; processed meats, 20 percent; and frozen food, 2 percent. Bryan (11) characterized C. perfringens food-borne illness as usually caused by meat or poultry which has been heated, and sometimes reheated, and held at room temperature for several hours. Such unsanitary practice rarely occurs in a commercial meat processing situation. Greenberg, et al. (35) reported the isolation of 19,727 putrefactive anaerobic sporeformers (PAS) from 2,358 samples of raw chicken, beef and pork in processing plants. The mean level of contamination was 2.8 PAS per gram of meat. The low incidence of these organisms in refrigerated meat is probably due to the fact that there are relatively

few psychrophilic Clostridium species, and those that are psychrotolerant grow quite slowly (46).

Beef, Pork and Comminuted Meats

Modern methods of processing meat utilize rapid cooling of carcasses to retard spoilage and the growth of pathogens. Such treatment is selective for psychrophiles and the predominant microflora of spoiled meat are gram-negative bacteria of the genus Pseudomonas. Ingram and Dainty (46) reported that many Achromobacter organisms which frequently have been associated with spoiled meats are regarded now as non-pigmented pseudomonads and the remainder are classified as Alcaligenes or Acinetobacter. These organisms are not considered actively proteolytic, nor do they attack amino acids to any degree.

Jay (49) reported that 56 percent of the organisms isolated from fresh and spoiled ground beef were Pseudomonas species. Only Pseudomonas, Achromobacter, Aeromonas and Flavobacterium were isolated from the spoiled beef, Pseudomonas representing 84.2 percent of the total strains isolated. Brown and Widemann (10) found that of 189 psychrophilic bacteria isolated from chilled beef and associated sources, 128 were typical pseudomonads, while only seven were gram-positive. Four of the seven gram-positive cultures were found to be Corynebacterium species.

Kraft, et al. (55), in a study of the incidence of microorganisms on turkey giblets, liquid egg and meat, found significant numbers of Microbacterium in fresh beef. Raw ground beef was reported by Casman, McCoy and Brandly (14) as unable to support growth of S. aureus. When the surface of raw beef was inoculated, however, excellent growth was obtained.

Comminuted meats such as bologna and other processed meats are frequently prepared in the same processing plants as fresh raw meat. Kempton and Bobier (52) found that the spoilage of vacuum packed luncheon meats was not related to the total count of the freshly processed meats, since the only organisms that multiplied in the presence of curing agents and under conditions of reduced air and low temperature were the lactic acid bacteria. Most of these products are cooked, destroying the lactic acid bacteria and most other vegetative cells, thus spoilage of the finished product was attributed to recontamination during slicing and packaging. Mol, et al. (63) confirmed that the lactobacilli and micrococci found in freshly manufactured cooked meats came mainly from the slicing and packaging lines, and recommended that cooked meats be handled and sliced in an area strictly separated from raw and cured meats.

Patterson (69) found that a majority of the 164 strains of staphylococci and micrococci isolated from fresh pork, curing pickle and maturing bacon were able to grow at 4 C and were tolerant to high

salt concentrations, a tolerance increased by the presence of nitrates commonly used in curing and preserving such products. Many of the original contaminants of the pork sides, Patterson concluded, could survive the curing process. Thus, the effectiveness of processing sanitation takes on added significance.

Goepfert and Chung (32) found that Salmonella inoculated on sliced luncheon meats were able to survive protracted periods of storage at 5 C, and were able to proliferate within vacuum sealed pouches at room temperature (23 C) in 48 hours. Such inocula, they reported, could arise through contact with an inadequately cleaned slicing machine, although reasonable attention to the sanitation of such equipment should prevent the occurrence of high initial loads, and the considerable growth necessary to produce symptoms of the disease when the meat is consumed.

Weissman and Carpenter (101) reported that the incidence of Salmonella in 50 pork carcasses was 56 percent and 74 percent in 50 beef carcasses examined in five processing plants in Georgia. Thirty-eight percent of fresh pork sausage examined in the same plants contained Salmonella. The authors noted that incidence of Salmonella did not vary appreciably from one area to another on the carcasses, indicating uniform distribution in the fresh meat, probably during processing.

E. coli, coliforms and enterococci have long been recognized

as indicators of fecal contamination in food products (58, p. 88-95; 81, p. 131-141; 83, p. 113-131). Insalata, Witzeman and Sunga (48) found that enterococci were recovered from more samples of processed foods than either coliforms or E. coli, and that frozen foods had the highest occurrence for both fecal streptococci and coliforms. Lörincz and Incze (59) proposed that Streptococcus faecalis replace E. coli as an indicator of possible fecal contamination in preserved meat products. The authors observed that the heat and cold resistance of S. faecalis is greater than that of E. coli, thereby making that organism a better indicator for meat products which undergo heat treatment and cold storage.

Poultry Contamination

The microflora of spoiled poultry is similar to that previously described for beef and pork. Walker and Ayres (94) described the chief spoilage organisms isolated from eviscerated poultry stored at 4.4 C in polyethylene bags as members of the Pseudomonas - Achromobacter group. Clark (17) characterized the type of growth prevalent on poultry skin as affected by scalding temperature. Pseudomonas grew best on skin scalded at 59 C, whereas Achromobacter grew best on unscalded skin. Scalding of birds immediately after killing makes the feathers easier to pluck, and removes the outermost layer of skin; the amount of skin removed being a function

of scald water temperature. Gunderson, et al. (36, p. 12) described immersion of birds in scald water contaminated with blood, feathers, feces, dirt, bacteria and other debris as a major source of contamination and recommended the use of spray type scalders.

Clark (18) compared the growth rates of psychrotolerant pseudomonads and Achromobacter species in five different tissues: skin, breast muscle, breast muscle lining, leg muscle, and gut cavity lining. The growth rate was fastest on skin, probably due to a shorter lag phase on that substrate. Skin is the most likely area to be contaminated during the handling of the carcasses, and the most difficult to sanitize once it has been contaminated (36, p. 29).

Barnes and Schrimpton (4) demonstrated that spoilage of uneviscerated poultry stored at 15 C was due to fecal streptococci, coli-aerogenes organisms, lactobacilli and clostridia. Walker and Ayres (94) found that Flavobacterium and micrococci comprised nearly 25 percent of the initial population of freshly processed poultry, but that their numbers decreased to almost zero at the end of 12 days storage at 4.4 C. Yeasts were the only organisms beside the Pseudomonas - Achromobacter group which flourished during the storage period, increasing from 1,000 per cm^2 to approximately 100,000 per cm^2 skin surface after two weeks. Walker and Ayres (93) reported that the yeasts frequently associated with poultry were Rhodotorula, Torulopsis, Saccharomyces and Candida. Walker and

Ayres in another study (95) reported that the number of coliforms, fecal streptococci and staphylococci isolated from turkeys were similar to those found in chickens, although generally, greater numbers per cm^2 were recovered from turkey skin.

Barnes and Impey (3) found Pseudomonas and Acinetobacter strains to be the most common organisms on poultry carcasses stored at 1 C.

The method of post processing chilling and storage affects the survival and activity of spoilage organisms on meat. Kotula, Thompson and Kinner (54) found that numbers of bacteria in the water in chill tanks increased, and the numbers on breast skin surfaces increased significantly during a six-hour chilling period. Brewer et al. (8) also observed a steady build up of total bacteria in continuous chillers and chill vats, but could not correlate the bacterial numbers in the chill tanks and those on the skin of birds during shelf life. The authors also noted that certain species originally on the birds were reduced nearly 100 percent during chilling or storage, but they were not the organisms involved in spoilage.

Patterson (70) reported that cooked eviscerated carcasses carried more fecal streptococci than uneviscerated and frozen eviscerated carcasses, confirming studies described earlier which indicate low survival rates for virtually all vegetative cells except the Pseudomonas - Achromobacter group and yeasts.

Rey and Kraft (72) found fluorescent pigment production, proteolytic and lipolytic spoilage of chicken stored at 5 C to be directly related to the availability of oxygen provided by the packaging procedure. Chickens frozen and then defrosted and refrigerated had a higher proportion of biochemically active psychrophiles on the surface of the meat than did freshly refrigerated chickens. Such storage is common practice in current marketing procedures. Heavy initial contamination with these psychrophiles leads to a significant reduction in shelf life.

Georgala and Hurst (31) described the presence of food poisoning bacteria in frozen foods as survival without growth. The major problem is the presence of the pathogens in the product before freezing; some can survive.

The greatest concern to poultry processors is Salmonella contamination. Studies performed by Kaufmann and Feeley (51) and Morris et al. (65) indicated a high level of Salmonella contamination in commercial chicken raising plants. Their studies indicated that poultry meal fed to the birds was the primary route of infection. The poultry meal consisted of rendered offal from processing plants associated with the poultry raising operations; the same offal which was removed from the birds on evisceration lines within the processing plants. Morris and Wells (64) found that 14.2 percent of the samples they tested in a poultry raising plant contained Salmonella.

They indicated that contamination was reduced by washing procedures in the plant but that the carcasses became recontaminated during evisceration and chilling. Woodburn (107) found that 27 percent of broiler-fryer type chickens purchased in retail stores in Indiana were contaminated with Salmonella. Equal numbers of both dressed whole and cut up chickens were contaminated.

Bryan, Ayres and Kraft (13) reported the incidence of Salmonella in two commercial turkey processing plants which further processed chilled, eviscerated and thawed carcasses into turkey rolls, roasts and similar products, both cooked and uncooked. Salmonella were isolated from 12 percent of chilled, eviscerated carcasses, 27 percent of finished products, and 24 percent of the processing equipment tested. Thirty-one percent of rinse samples taken from hands and gloves of processing personnel were positive for Salmonella. Recoveries of Salmonella were higher on days when freshly killed turkeys were processed than when frozen-defrosted carcasses were processed.

Salmonella derby, an organism tested in the germicide study in this thesis, was isolated in the above work (13) from chilled eviscerated turkey carcasses, processing equipment and finished (uncooked) product. Morris and Wells (64) found the same serotype in feces, equipment and housing of chickens, and Morris, et al. (65) recovered S. derby from the excreta of chicks.

Isolation Procedures

The recovery of bacterial contamination from meat and poultry processing plants involves several problems; different techniques must be applied for evaluation of surface contamination (metal surfaces, equipment, gloves, etc.) than can be utilized for recovery of organisms from meat and skin. Care must be taken to select representative samples from processing equipment and products. The method of processing and preserving meat, as well as the type of product examined must be considered in the selection of recovery media and temperature of incubation.

Standard Methods

"Reference Methods for the Microbiological Examination of Foods" (67) represents a recent effort to standardize the criteria and techniques utilized by microbiologists in the food industry. This report describes the problems faced by the food microbiologist when trying to determine the correct procedure for evaluating contamination levels. Methods for the detection of Salmonella are compared from several sources such as the "Bacteriological Analytical Manual" (91), "Examination of Foods for Enteropathogenic and Indicator Bacteria" (58), "Recommended Methods for the Microbiological Examination of Foods" (81), and several other authoritative

references. The comparison makes clear that, while most methods are similar, significant differences exist, and universally acceptable standard methods are needed.

Microbiological Sampling of Surfaces

Wilkinson (103) reported that the survival of bacteria on metal surfaces was closely related to relative humidity and the species involved. The meat-metal contact surfaces in processing plants are usually wet during processing operations, representing nearly ideal conditions for survival of bacterial contamination.

Favero, et al. (26) summarized the four basic methods used in microbial sampling of surfaces. No single assay procedure was considered capable of completely recovering all microbial elements on a surface. The rinse technique was found most accurate for enumerating viable microorganisms, and the direct surface agar plating technique was recommended for enumerating particles containing viable microorganisms.

Fincher (28) described in detail the methods currently favored for surface sampling and noted that the APHA swab-rinse method was the most widely used. This method requires that the microbial suspension be plated at several dilutions in suitable growth medium. Clark (16) reported that the spread plate method of inoculation yielded significantly higher counts (70-80 percent) than the pour plate method.

The author recommended, in a more recent study (19), that predrying plates before use permitted larger inoculations with equally good recovery.

Mossel and Moosdijk (66) found no significant difference between the two methods of counting in 90 percent of the samples studied. The authors concluded that the slightly higher spread plate counts in the remaining instances could be accounted for by the increased disruption of bacterial conglomerates commonly observed in that procedure.

In addition to the standard methods for evaluation of contamination on meat and skin surfaces in previously mentioned references, many adaptations and innovative techniques have been described in the literature. Such variations include skin blending (2), direct microscopic examination of membrane filters (105), direct harvesting of wash diluent (104) and infrared estimation of microbial population on chicken skin (76).

Many factors affect the recovery of contamination from the plant environment. The scalding operation exposes bacteria on skin surfaces of poultry to temperatures of approximately 60 C (36, p. 12). Beuchat and Lechowich (6) reported that the incubation time for maximum recovery of S. faecalis cells exposed to 60 C was significantly longer than for unheated cells.

In another study (5) the authors reported that, upon exposure to

heat, S. faecalis cells became sensitive to increased salt concentrations.

Bacteria exposed to sanitizers may require special recovery media and conditions to differentiate between those cells which are injured but viable, and those which are dead (79, 80).

Identification of Isolates

Specific details concerning differentiation of types of organisms are described in the "Manual of Microbiological Methods" (84) and "Bergey's Manual of Determinative Bacteriology" (7). In addition, other taxonomic schemes have been devised which aid in classifying specific groups of organisms. Cowan and Steel (20) developed diagnostic tables for the common medical bacteria which utilize nine physical and biochemical criteria to characterize most organisms to the genus level. One of the most useful of those is the glucose fermentative versus oxidative metabolism test of Hugh and Leifson (43).

The classification of the gram-negative bacteria associated with meat spoilage is currently undergoing revision. Until recently, many investigators differentiated Achromobacter and Alcaligenes from pigmented and non-pigmented pseudomonads by the criteria described in "Bergey's Manual" (90). There has been increasing evidence in the literature of acceptance of a different set of criteria (47, 89), wherein

strictly oxidative pigmented and non-pigmented rods which exhibit polar flagella are classified as Pseudomonas, while peritrichous non-pigmented species remain as Achromobacter. A new genus, Acinetobacter, was proposed to include the nonmotile very short or coccoid rods often occurring in pairs which had formerly been grouped with the motile Achromobacter species. The position of Alcaligenes and Flavobacterium in this new system is uncertain, and biochemical and morphological features remain to be clearly defined.

Other studies are available in the literature which deal more specifically with the taxonomy of Pseudomonas (85), aerobic sporeformers (106), and the yeasts (56, 96, 102).

Germicides

Evolution of Modern Germicides

Germicides evolved from compounds which were found effective in controlling foul odors in sewage and garbage, long before microorganisms were discovered and recognized as the causative agents of putrefaction. The association between foul odors and disease led to the use of disinfecting solutions prior to the establishment of the germ theory of human disease. But dramatic results achieved early in the 19th century with the use of chloride of lime, carbolic acid (phenol), tinctures of iodine and similar compounds went ignored, for the most

part, for many years (57, p. 3-8).

It was not until 1867 when Lister (9, p. 83-85) reported his successful techniques for the prevention of wound infections by soaking link dressings with phenol that general acceptance of aseptic procedures began. When Robert Koch (9, p. 101-108) developed the methods of modern bacteriology in 1881, the significance and potential of germicides and disinfection were finally recognized, and rapid progress followed.

Chlorine

Henry first produced chloride of lime ($CaOCl_2$) near the end of the 18th century. Fifty years later the first Royal Sewage Commission of Great Britain reported the use of chloride of lime for deodorizing London sewage (37, p. 465). Semmelweis (9, p. 80-82) had reported success prior to that time in controlling the spread of human infection by having doctors wash their hands in chloride of lime solutions. Watt, in 1851, prepared sodium hypochlorite by electrolyzing a solution of sodium chloride, and in 1881 Koch reported the first instance in which hypochlorites were used distinctly for the purpose of destroying microorganisms (37, p. 465). In 1894 Traube established the use of hypochlorites as purifying and disinfecting agents in water supplies. Hypochlorites came into extensive use as disinfectants during World War I and today are used in a wide range of

applications as disinfectants, sanitizers and germicides (57, p. 278).

Chemical and Physical Properties

Hypochlorites are usually marketed as powders containing calcium hypochlorite and sodium hypochlorite combined with hydrated trisodium phosphate, and as liquid sodium hypochlorite solutions (37, p. 469).

The stability of hypochlorite in solution is affected by temperature, concentration and pH, the latter being the most important. According to Sykes (87, p. 382) solutions of about pH 9.5 are the most stable. More concentrated solutions are less stable than weaker ones, and all hypochlorites are sensitive to heat and light.

Hypochlorites are formed by the reaction of molecular chlorine with water according to the following reaction:

(1) $Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$ $\qquad K = 5 \times 10^{-14}$

Hypochlorous acid (HOCl) dissociates in water, the degree of dissociation being determined by the pH of the solution.

(2) $HOCl \rightleftharpoons H^+ + OCl^-$ $\qquad K = 2.95 \times 10^{-8}$

Oxygen may also be liberated from hypochlorous acid under certain conditions by the following reaction:

(3) $2HOCl \longrightarrow 2HCl + O_2$

The free oxygen formed by reaction (3) above may also contribute to the effectiveness of hypochlorite as a germicidal agent (42, p. 40-45). The bactericidal efficiency of hypochlorite is closely paralleled with the hypochlorous acid concentration, which increases with decreasing pH. Alkaline solutions of hypochlorites, in which OCl^- is far in excess of HOCl definitely do possess germicidal properties. This may possibly be because as traces of HOCl are consumed in the bactericidal process, the hydrolysis equilibrium (equation 2) will shift to the left and HOCl will continuously be formed to carry on the germicidal action.

Chlorine is available from various forms found in hypochlorite solutions in water: hypochlorous acid (HOCl), hypochlorite ion (OCl^-) and elemental chlorine (Cl_2). Because of its electronic configuration, there is a strong tendency for chlorine to acquire extra electrons and change to inorganic chloride ions, making it a very strong oxidizing agent. This property is believed to account for its high germicidal activity.

Chlorine is very corrosive, readily attacks metals and is a strong irritant to tissues. Chlorine also attacks inorganic and organic impurities in water, and in the presence of ammonia and other nitrogenous matter forms chloramines or N-chloro compounds. These compounds are called combined available chlorines and possess significantly less germicidal capacities than the free available chlorines.

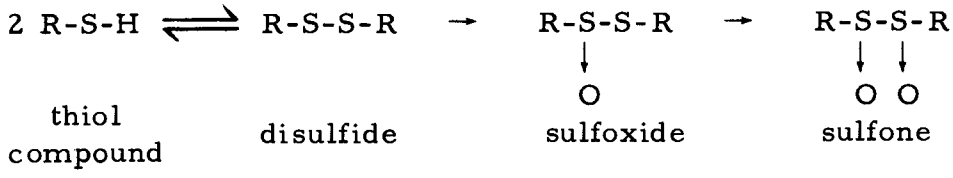
The free available chlorine and combined available chlorine are collectively described in water as total residual (available) chlorine.

In the presence of large quantities of organic material, most or all of available chlorine applied may be consumed, imparting a high chlorine demand on the germicide solution. In practical use, sufficient chlorine must be made available to overcome the chlorine demand and leave a residual of available chlorine to maintain germicidal activity, a condition referred to as break-point chlorination (57, p. 278-294).

Mechanism of Action

Chlorine reacts with sulfhydryl groups on cellular proteins and is an extremely active inhibitor of sulfhydryl enzymes. It is generally accepted that this inhibition causes cell death by interruption of essential metabolic systems, termination of glucose oxidation and loss of cell viability (42, p. 40-45).

The sequence of oxidation of the thiol compounds is as follows:



Oxidation beyond the disulfide stage is practically irreversible, although mildly reducing conditions at the cellular level may reverse

inhibition prior to that point (44, p. 50).

In addition, hypochlorite solutions attack proteins and related compounds, forming derivatives in which chlorine is linked to nitrogen. α amino acids are chlorinated to chloramino acids which further decompose to aldehydes or ketones, ammonia, carbonic acid and sodium chloride (42, p. 40-45).

Iodine

Iodine was used in medicine more than 150 years ago for the treatment of goiter. It was first applied in the treatment of wounds as early as 1839 and was used for that purpose in the American Civil War. The most common form was a solution of iodine in alcohol, usually 5 percent. In 1873 Davine found that iodine solutions would attenuate the virulence of anthrax bacilli and later reported that high dilutions could destroy anthrax in blood in a period of one hour.

Iodine has been used in many forms as a skin antiseptic, a disinfectant for wounds and mucous surfaces and as a general disinfectant and germicide in air, water and on surfaces (57, p. 329). The limitations of iodine prevented its use as a common germicide-sanitizer until recently, when iodine was mixed with surface active agents to form iodophors. These compounds retain the germicidal properties of iodine while eliminating many of its disadvantages, and have come into extensive use as detergent sanitizers in hospitals,

and in the dairy and food industries (74, p. 143).

Chemical and Physical Properties

Iodine is a solid at room temperature. It is only slightly soluble in water and its solubility is increased in the presence of iodide ions; it is very soluble in alcohol.

In water, iodine undergoes the following reactions:

(1) $I_2 + H_2O \rightleftharpoons HOI + I^- + H^+$ $\quad K = 0.3 \times 10^{-12}$

Hypoiodous acid dissociates, forming hypoiodite ion:

(2) $HOI \rightleftharpoons H^+ + OI^-$ $\quad K = 5 \times 10^{-13}$

Periodides are formed from iodine in the presence of iodide ion.

(3) $I_2 + I^- \rightleftharpoons I_3^-$ $\quad K = 1.4 \times 10^{-3}$

Hypoiodous acid may decompose, yielding iodate and iodide.

$$3HOI \rightarrow HIO_3 + 2H^+ + 2I^-$$

At pH values below 6.0 iodine is principally in the form of I_2, and it is within this range where maximal germicidal effectiveness is observed. As the pH is raised, the concentration of hypoiodous acid increases and a marked decrease in killing rate occurs. The germicidal activity of the triiodide ion is 0.08 that of I_2. The

bactericidal capacity of iodide ion is negligible (42, p. 45).

Iodine solutions are brown, and stain strongly. Iodine skin disinfectants are usually aqueous or alcoholic solutions containing low concentrations of both iodine and potassium iodide. The aqueous solutions are less irritating to tissue than are the tinctures, but iodine can be harsh and can even cause severe blistering if used carelessly.

The advantages of iodine are its high germicidal activity, its lack of selectivity against different types of bacteria, and its bactericidal rather than bacteriostatic action (87, p. 400-409). The concentration of iodine necessary to destroy bacteria does not vary greatly with different species of organisms (62, p. 174).

Iodophors

Iodophors are combinations of iodine and a solubilizing agent or carrier in which the resulting complex contains and slowly releases free iodine when diluted in water. The formulations of these solutions are controlled by the use of phosphoric acid to maintain a preferential pH through the normal dilution range. The carrier is usually a non-ionic surface active agent, although iodophors consisting of iodine plus cationic surface active agent are also available (57, p. 334-337).

In solution, iodophors are able to solubilize up to nearly 30 percent of their weight in iodine, of which 70 to 80 percent may be

released as available iodine when the concentrated solution is diluted. The iodine is bound in the form of micellar aggregates which are dispersed upon dilution as the linkage of the iodine progressively weakens. When diluted beyond the minimum micellar concentration, an iodophor may be considered as a simple aqueous solution of iodine. Some of the iodine may eventually convert to inorganic iodide, but sufficient free iodine is present to retain strong germicidal activity.

Iodophors have eliminated most of the disadvantages of aqueous or alcoholic iodine solutions, and have many practical advantages over other bactericides. They are strong germicides, yet are almost odorless, stable in storage, relatively non-toxic, almost non-irritating and do not sensitize humans.

Iodophors are effective bactericides over a wide temperature and pH range; yet, because their activity is that of the free iodine present, they retain the advantages of the simple iodine solutions described earlier. Organisms do not develop a resistance to iodine.

Iodine is affected by organic matter to a lesser degree than chlorine. When iodine has been used up by organic matter, the yellow-brown color of the solution disappears, the solution acting as its own automatic indicator of potency. Iodophors do not stain skin or metals (except silver), but do stain soiled matter such as milk solids, milk stone and blood, and automatically indicate if a surface is clean. Iodophors are surface active and possess strong wetting powers, thus,

they are detergents as well as germicides, one application performing two functions.

If improperly used (e.g., in excessive concentrations or with very hot water), iodophors may be corrosive and stain skin surfaces (21).

Mechanism of Action

The germicidal effects of iodine are believed to be the result of both direct iodination and oxidation of sulfhydryl groups on proteins (42, p. 46). The correlation between germicidal efficiency and the free molecular iodine concentration suggests that direct intervention of iodine causes protein precipitation; hypoiodous acid has little or no effect on this process (87, p. 401).

Shikashio, Sandine and Elliker (82) studied the mechanism of action of iodophor on several sulfhydryl group dependent enzymes and reported that the activity of alcohol dehydrogenase and glucose -6- phosphate dehydrogenase which was inhibited by iodophor was completely restored by glutathione and cysteine respectively. Partial reversal of inhibition of lactic dehydrogenase by iodophor was effected by cysteine. The sulfhydryl-independent enzyme catalase was not inhibited by 60 ppm iodophor at pH 7.0. At pH 8.5, however, up to 60 percent inactivation was observed, indicating the probability of direct iodination reactions on the tyrosyl and possibly histidyl

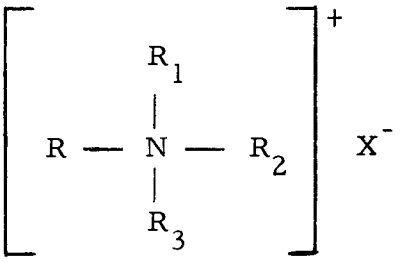
moieties of the enzyme. The authors concluded that, at least in part, the activity of iodophor was due to the oxidation of sulfhydryl groups located on proteins and other constituents essential for glucose oxidation and maintenance of cell viability.

Quaternary Ammonium Compounds

Quaternary ammonium compounds (QACs) are surface active cationic quaternary ammonium germicides which are basically organically substituted ammonium compounds. These compounds were first synthesized shortly after the beginning of the 20th century, but it was not until Domagk reported their use as effective antiseptics and disinfectants in 1935 that these compounds came into wide use. Since that time many variations of these cationic detergents have been synthesized and over 1,000 publications on the subject have appeared. The effectiveness of this class of germicides was exaggerated in the early literature due to properties which made laboratory evaluation difficult and deceptive (87, p. 362-363).

Chemical and Physical Properties

QACs may be considered an organic nitrogen compound in which the nitrogen has a covalence of five. They follow the general configuration:



The X represents a halogen, hydroxyl or alkoxy radical, and the R represents an alkyl, aryl-alkyl or heterocyclic radical (57, p. 431). One of the R substituents is an alkyl group containing C_8 to C_{18} carbon chain lengths. The surface activity of these compounds is mainly due to these longer chain groups which are derived from natural vegetable or animal oils, mineral oils and resins.

QACs are cationic surface active agents in which the lipophilic group is bound to the nitrogen nucleus, and upon dissociation in water the long chain part of the molecule is positively charged.

Anionic surface active agents interfere with the germicidal activity of QACs. No antagonism is observed in the presence of non-ionic surface active agents, since the latter do not ionize in aqueous or other solvents. Because some of the non-ionic compounds are excellent solubilizing and foaming agents, they are often used in formulations with QACs to enhance solubility and improve germicidal-detergent activity.

The QACs have several inherent features that make them desirable germicides. They contain no phenol, iodine, active

chlorine, mercury or other heavy metals. They have no tendency to decompose in storage, are non-toxic when used in the proper concentrations, and are colorless and odorless. They are effective against a wide variety of organisms. When left on surfaces, QACs exhibit a marked bacteriostatic activity over a long period of time, and are less susceptible to inactivation by organic matter than are the halogen germicides.

Because of their cationic nature QACs are inactivated by soaps (anionic) which limit their practical applications. Cations normally responsible for the hardness of water, such as calcium and magnesium, reduce the germicidal activity of QACs (57, p. 430-446). Generally, QACs are more selectively active against gram-positive organisms than against gram-negative types (87, p. 366). Other workers have found organisms, notably Pseudomonas species, which are highly resistant to QACs and apparently decompose them (60, 98).

Mechanism of Action

Early workers attributed the antimicrobial activity of QACs to their high surface activity. It was believed that absorption to the bacterial cell wall caused its disruption and subsequent leakage of cell contents. In very low concentrations surface active agents precipitate proteins and also dissociate conjugated proteins. In higher concentration, and in the presence of concentrated cell suspensions,

membrane disruption and loss of purines, pyrimidines, inorganic phosphorous, and amino acids from the cell may be significant factors in the germicidal action of QACs. Lethal concentrations of QAC cause complete and irreversible inhibition of bacterial oxidase and dehydrogenase systems. The inhibition of those enzymes may be responsible for increased permeability, metabolic inhibition and cell death (42, p. 69-71).

More recent studies were based on the observation that low or sublethal concentrations of QACs actually stimulated respiration. It was noted that in baker's yeast, uptake of oxygen and output of CO_2 were stimulated by benzalkonium chloride under aerobic conditions. In low concentrations of the QAC, no membrane damage or loss of cell contents were observed. Higher concentrations caused cell lysis. A correlation was observed between loss of potassium (membrane disruption), inhibition of glucose fermentation, and appearance of pyruvate decarboxylase activity, but no inhibition was observed in individual enzymes. In studies on aerobic yeast cells in the presence of excess glucose, benzalkonium was found to block the oxidation of acetate in the Krebs cycle, causing an accumulation of acetate. The conclusion was reached that the acetaldehyde $\rightleftharpoons$ acetate or the acetate - CoA reaction must be the principle site of QAC inhibition (44, p. 44-47).

Germicide Evaluation

In 1897 Kronig and Paul (9, p. 163-175) first established methods and criteria for the evaluation of chemical disinfecting agents. The principles they described are fundamentally similar to those applied today. Among the criteria they proposed were the use of standardized culture suspensions, germicide solutions of equal strength, and careful maintenance of uniform testing conditions such as growth media, temperature and recovery conditions. Special emphasis was placed upon the removal or inactivation of disinfecting solutions following the exposure period. Since that time other procedures have been developed which established recognized standards with which the germicidal efficiency of nearly all chemical disinfectants could be compared. Rideal and Walker (73, p. 31) first proposed the use of phenol as a reference standard in 1903, because at that time phenol was available in pure form and was widely used as a disinfecting agent. A standard test organism, Salmonella typhosa, was used for this test because it was considered the most important human pathogen at the time. From this origin many modifications have been made which established the phenol coefficient as the standard method of evaluating germicidal efficiency. This procedure is designed to determine the highest dilution of a germicide which will kill the test organism within a series of time intervals under specified

conditions. When these values are compared to those obtained for phenol, specific calculations yield a phenol coefficient number.

This system has limited practical application, however, because the maximum dilution found to kill in this test does not necessarily provide disinfection in applications which are usually recommended. The indirect information obtained from the phenol coefficient test is further limited by restricting the variety of conditions under which many different types of germicides are most efficient (57, p. 133-158).

Current Methods

In the past 25 years several methods have been proposed which more accurately evaluate those germicidal chemicals used to cleanse equipment and utensils in dairies and food plants and which are commonly referred to as sanitizers. The purpose of these agents is to reduce the number of bacterial contaminants to a safe or relatively safe level, while not necessarily disinfecting--that is, destroying infectious agents.

In 1948 Weber and Black (99) proposed a laboratory procedure to evaluate germicides used in sanitizing food utensils. The method involves a dilution tube procedure which yields a high correlation between test results and those found in actual use.

Chambers (15) revised this method in 1956, and this modified

procedure is now used commonly by regulatory agencies in determining the effects of hard water on germicides and sanitizers where speed of action is essential, such as use in food plants, restaurants and dairies.

An excellent description of these and other procedures recommended for the evaluation of germicides and sanitizers may be found in a review by Shaffer and Stewart (57, p. 159-178).

Practical Applications of Germicides

Much of the earlier work with sanitizers was focused on their application in the dairy industry (24, 25, 68). The interest of the food industry in effective sanitation programs has accelerated in recent years, notably due to expanding and increasingly stringent governmental inspection programs, improved procedures for bacteriological sampling of foods, and widening public awareness of the public health hazard aspect of food on today's market. This interest is reflected also by the number of international conferences which have been recently held for the purpose of establishing microbiological standards for foods (30).

Goldenberg and Relf (33) noted that the rapid growth of prepacking in all sections of the food industry has diverted emphasis away from the use of disinfectant at the retail level and toward the processing aspect. There is increasing evidence in the recent

literature of successful application of germicides in the food processing environment. Chlorination of final wash water (75) and chill tank water (71) have proved highly effective in reducing total counts, and particularly Salmonella contamination (23, 92) on eviscerated chickens. Similar results were reported for chlorinated hot water plus detergent in cleaning surfaces and equipment in a bacon factory (22).

MATERIALS AND METHODS

Isolation and Identification of Meat Microflora

Isolation Procedures

Bacteriological examinations were carried out on three occasions in a turkey processing plant and in one visit to a beef processing plant. Representative samples were taken from processing equipment and tools at various points along evisceration lines, storage areas, chill tanks, packaging areas, and from the carcasses directly. At several points samples were taken just before and immediately following routine washing procedures.

Samples were collected from uneven surfaces by the swab contact method (97, p. 146-147) using both plain phosphate buffered rinse solutions and buffered rinse solutions containing 0.05 percent sodium thiosulfate to neutralize any residual chlorine present in the samples. Flat surfaces were sampled with Rodac plates (97, p. 249) prepared with standard methods (SM) agar (97, p. 232-233) supplemented with 0.5 percent Tween 80 and 0.07 percent lecithin, as recommended by Fincher (28). "Pre-Med" contact plates[1] were also used to test flat surfaces.

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1. Hyland, Division Travenol Laboratories, Inc., Los Angeles, CA 90039.

The purpose of the examinations was to collect representative samples of equipment and product microflora, and no specific effort was made to enumerate the organisms found, other than to ascertain relative levels of contamination from different sources. Salmonella cultures were collected and isolated according to APHA standard methods (81).

Identification Procedures

Swab rinse solutions were returned to the laboratory packed in ice and diluted in 99 ml saline blanks (81) and inoculated by the pour plate method (97, p. 34-52). Duplicate plates were incubated at 30 C and 37 C for 48 hours, and at 7 C for 7 to 14 days. With the exception of the Salmonella cultures, selective media was not employed to obtain specific types of bacteria or yeast; SM agar was used in all cases.

Morphologically distinguishable colonies were picked from the incubated pour and contact plates and subcultured in SM broth and agar. Cultures were identified by the method of Cowan and Steel (20) and those described by Thornley, Ingram and Barnes (90). Further tests on several gram-negative organisms were carried out using the Enterotube system (61). Identification of most of the organisms recovered was carried out to the genus level, with the exception of the yeasts.

Bacteria used in the germicide study were further identified to species by the criteria of "Bergey's Manual of Determinative Bacteriology" (7). The Salmonella culture was identified to the genus level according to methods recommended by the Public Health Service (58, p. 100-113), and was serotyped by the USDA Animal Disease Laboratory, Phoenix, Arizona.[2] The yeast culture was identified according to procedures in "The Yeasts" (96).

Cultures Used in Germicide Tests

Selection

Representative isolates were selected for use in the germicide studies on the basis of their significance as meat and poultry microflora as reported in the literature. Salmonella was chosen due to its prevalence as a foodborne pathogen, particularly in poultry. Spores of Bacillus licheniformis are characteristically resistant to germicides and are widely distributed in soil and food (46, p. 620). A member of the genus Candida was selected as a typical food spoilage yeast. Pseudomonas aeruginosa and Micrococcus luteus were selected because they were encountered frequently both in meat and on equipment. Streptococcus faecalis and Escherichia coli are well recognized indicators of fecal contamination in food and water.

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2. State-Federal Animal Disease Laboratory, 322 Capitol Annex West, 1688 West Adams, Phoenix, Arizona 85007.

Preparation of Cultures for Germicide Tests

One liter of Schaeffer's sporulation medium (78) was inoculated with Bacillus licheniformis culture and incubated on a shaker for six days at 30 C. The resulting spore suspension was washed three times with physiological saline, heat shocked at 80 C for ten minutes, and diluted with physiological saline to obtain the desired concentration as determined by standard plating procedures. The spore suspensions were aseptically tubed in quantities sufficient for each germicide test, stored at 4 C, and heat shocked again immediately prior to use.

The other bacterial and yeast cultures were prepared in the following manner: 18 to 24 hr SM broth cultures of the isolates were washed onto medicine bottle slants prepared with SM agar, and the slants were drained and incubated at the optimum temperature for the culture. The 24 hr growth was washed from the slant with 20 ml sterile 0.85 percent saline, aseptically filtered through Whatman number 2 paper to break up clumps of cells, and immediately placed in an ice water bath. A one ml aliquot of the suspension was used to make a 1:10 dilution in physiological saline, and the O.D. was measured at 625 nm. The suspension was diluted as necessary to obtain a predetermined O.D. for each culture in order to achieve the desired concentration.

Germicides and Titrations

Germicides

Four germicides were tested in the study: a sodium hypochlorite solution sold under the brand name of XY-12, a dimethyl dichlorobenzyl ammonium chloride sold as Ster-Bac (Formula KQ-10), and two iodophors. Iodophor A was a butoxy polypropoxy polyethoxy ethanol-iodine complex containing 6.5 percent phosphoric acid, and iodophor B was a nonyl phenoxy poly (ethyleneoxy) ethanol-iodine complex containing 25 percent phosphoric acid, sold as Iodophor DF and Iodophor ID-10 respectively.[3]

Titrations

Stock solutions of approximately 1,000 ppm of each germicide were prepared by diluting the original solutions with tap water. Within 24 hr of each germicide test the iodophor and hypochlorite stock solutions were titrated to determine their actual concentration. The available chlorine of the hypochlorite stock solution was determined by titration with standard sodium thiosulfate by the iodometric method (1, p. 83-85). Available iodine of the iodophor stock solutions was determined by titration with standard thiosulfate to a colorless endpoint.

The stability of QAC compounds is such that concentrations do

3. Klenzade Products, Division of Economics Laboratory, Inc., New York.

not vary significantly over a period of years, and stock solution concentrations were based on accurate dilution of the original solution, which was assayed by the method of Furlong and Elliker (29). The concentration was determined again following the completion of the germicide tests two years later, and was found to be the same.

Final Germicide Solutions

On the basis of the concentration of the stock solutions, the amount of stock solution required in 100 ml total solution was calculated and added to the reaction flasks immediately before each germicide test. The final germicide diluent was either sterile distilled water ("soft" water), or sterile distilled water to which were added sterile salt solutions necessary to reach a water hardness of 500 ppm $CaCO_3$ (27).

Acidified hypochlorite solutions were used in several sporicide studies and were prepared by using 0.25 M phosphate buffer (pH 7.0) as the germicide diluent.

Germicide Test Materials

Inactivation

Germicides were neutralized by the procedures suggested by Humphreys and Johns (45). Following the desired exposure period,

one ml of the organism-germicide reaction mixture was plated directly into a petri plate containing two ml of the appropriate inactivator. The plates were then poured with SM agar, mixed, and allowed to solidify.

Hypochlorite and iodophor germicide inactivator solutions containing 360 mg sodium thiosulfate and 40 ml 0.25 M phosphate buffer per liter were adjusted to pH 7.2 and sterilized. When high concentration (200 ppm) high phosphoric acid iodophors were tested against spores, the inactivators were prepared as above but with 720 mg sodium thiosulfate and 500 ml 0.25 M phosphate buffer per liter. The higher quantities of buffer were required to insure a rapid rise of pH to near neutrality upon inactivation of the germicide.

Quaternary ammonium compounds were neutralized with sterile inactivator solutions containing 2.222 g Azolectin (lecithin), 15.8 ml Tween 80, and 40 ml 0.25 M phosphate buffer diluted to 1 liter with distilled water. The pH was adjusted to 7.2. Plating media for QAC tests was SM agar to which 1 g Azolectin and 7 ml Tween 80 per liter were added before sterilization.

Germicide Effectiveness Tests

Test Methods and Controls

Germicide effectiveness studies were carried out by the method of Chambers (15) with several modifications.

One organism suspension was used for each test; the concentration was determined before and after the test by standard dilution and plating techniques. At least two different types of germicides at various concentrations were used against the organism suspension during each test. The germicides in each test were prepared in both hard and soft water. In this manner, the relative efficiency of each germicide could be evaluated under uniform conditions, and small but significant differences noted. All tests were performed at room temperature (23-25 C).

One additional control reaction flask was prepared for the highest concentration of each germicide tested, in both hard and soft water. One ml of this germicide was added to two ml of the appropriate inactivator in a petri plate, and a known dilution of the organism suspension which would yield a countable plate was then added within 15 seconds. Counts obtained in this manner were correlated with those obtained by standard plating of the known dilution and were used as inactivator effectiveness controls. Inactivator toxicity controls were carried out by adding the known dilution of culture suspension to duplicate plates containing two ml of the inactivator, pouring one plate immediately and the other after the longest period of exposure for that test. Significant difference between the known dilution count and inactivator exposed count would indicate inactivator toxicity. In no case was any inactivator toxicity noted.

Organisms were exposed to the germicide solutions for periods of 15, 30, 60, 120 and 300 seconds. Spores were exposed for 30 seconds, 2, 5, 10 and 20 minutes. All tests were performed in the absence of organic matter.

The pH of the soft and hard water, and control germicides were measured prior to each test. The pH of the organism germicide mixture was measured immediately following the last exposure period.

RESULTS

Contamination in a Turkey Processing Plant

The predominant microflora in various areas of the turkey processing plant examined are shown in Table 1. In this plant the time between killing the birds and placing the eviscerated carcasses in chill tanks was approximately 30 minutes. The temperature of the processing area was about 23 C. The carcasses were stored overnight in chill tanks containing an ice-water mixture, and spray rinsed, packaged and frozen on the following day. Water used throughout the plant was chlorinated at a level of 5 to 7 ppm.

The most heavily contaminated area of the plant was the giblet handling equipment on the evisceration line. This equipment consisted of conveyor belts, drums, and grinders which retained large quantities of skin, fat and muscle tissue on their surfaces which were difficult to remove without thoroughly dismantling and cleaning the machinery. The particulate matter provided a reservoir of microbial contamination which built up during operating hours and was transfered to the giblets, which were placed in the turkey cavities.

A broad range of organisms were found in the kill area where blood, feathers and fecal material covered much of the operating equipment and the workers. The predominant organisms in this area were the enteric bacteria, including Salmonella, and pseudomonads.

Table 1. Microbial contamination observed on different equipment surfaces and products in a turkey processing plant.

Microflora isolated	Surfaces examined				
	Kill area	Evisceration line equipment	Giblet handling equipment	Turkey carcass following evisceration	Packing area equipment
Achromobacter	+	-	++	++	-
Aeromonas	-	-	+	+	-
Alcaligenes	-	-	+	-	+
Aerobic sporeformers	-	-	-	+	-
Coliforms	++	-	++	++	+
Coryneforms	-	-	+	+	+
Flavobacterium	-	++	+	+	++
Micrococci	-	++	+	-	-
Pseudomonas	++	+	++	-	-
Proteus	++	-	-	-	-
Salmonella	+	-	-	-	-
Staphylococcus	-	-	+	+	+
Streptococcus	-	-	-	+	-
Yeasts	-	+	-	+	+

- Infrequently isolated or not found.

+ Frequently isolated but not predominant.

++ Frequently isolated and predominant organism(s) isolated from that source.

The skin of turkeys after evisceration also contained a wide spectrum of contaminants, but the overnight chilling process favored the survival only of psychrophiles. The low level of chlorine in the chill tank water was probably rapidly depleted by the carcasses and provided limited germicidal effect.

The majority of organisms in the packing area were those which survived the chilling process and proliferated on the packing equipment surfaces. Sharp edges in the area where the turkeys were placed in plastic wrappers contained a large amount of particulate matter which was heavily contaminated. Removal of this matter with plain cold water rinses significantly reduced the level of microbial contamination.

Contamination in a Beef Processing Plant

In contrast to the temperature of the poultry plant, the beef processing plant operating spaces were refrigerated in the range of 0 to 4 C. The exception was the kill floor, where the temperature was approximately 27 C. Most of the equipment and working surfaces in this plant contained relatively high levels of contamination in spite of the low ambient temperatures and the reported use of in-plant chlorination at a level of 200 ppm. The results of the examination of this plant are summarized in Table 2.

The most heavily contaminated surfaces were those held at the

Table 2. Microbial contamination observed on different equipment surfaces and products in a beef processing plant.

Microflora isolated	Surfaces examined				
	Kill floor equipment	Carcass cutting area	Meat boning equipment	Packing room	Meat on shipping floor
Achromobacter	-	+	++	++	++
Aeromonas	-	+	-	+	-
Aerobic sporeformers	+	+	-	+	-
Coliforms	+	+	-	-	-
Coryneforms	+	-	-	-	-
Flavobacterium	++	+	-	-	-
Micrococci	++	++	-	-	-
Pseudomonas	-	++	++	+	++
Staphylococcus	++	+	-	-	-
Streptococcus	+	-	-	-	-
Kurthia	-	++	-	-	-

- Infrequently isolated or not found.

+ Frequently isolated but not predominant.

++ Frequently isolated and predominant organism(s) isolated from that source.

lowest temperature (0 C). These surfaces were stainless steel trays, counters and rotary conveyors along which the cut up carcasses were trimmed and boned. At this point, the meat had been stored in the plant for several days.

This plant also produced lunch meats and sausage, some of which were sliced shortly before packing and shipping. The slicing and packaging machinery associated with these meats were found to be heavily contaminated, as were the final products.

The predominant microflora of this plant, particularly in the packing area, were members of the Pseudomonas - Achromobacter group. These organisms are most frequently associated with spoilage of fresh meats, and represent a significant economic problem for meat processors.

Germicide Effectiveness Studies

The destructive effect of several germicides on Salmonella derby, an organism isolated from the kill area of the turkey plant, is shown in Table 3. Low concentrations (6 ppm) of iodophor and hypochlorite germicides killed all cells within 30 seconds, and in most cases, within 15 seconds. USDA hard water (500 ppm $CaCO_3$) had an average pH of 8.87 before the addition of germicide. In these solutions, the pH of low concentrations of the acidic iodophors were raised above the level of maximum efficiency, yet their germicidal

Table 3. Destruction of Salmonella derby by iodophor, hypochlorite and QAC germicides.

Germicide	Conc.	Test water	pH of germicide solution	Average number of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
	ppm							
Iodophor A	6.0	distilled	3.75	0	0	0	0	0
	6.0	USDA[b]	7.59	15	6	0	0	0
Iodophor B	6.0	distilled	3.18	0	0	0	0	0
	6.0	USDA	6.64	58	0	0	0	0
QAC	100	distilled	6.66	0	0	0	0	0
	50		6.03	7	1	0	0	0
	100	USDA	8.66	TNC	9	0	0	0
	50		8.70	TNC	TNC	TNC	TNC	12
Hypochlorite	6.0	distilled	6.60	0	0	0	0	0
	6.0	USDA	8.71	0	0	0	0	0

a Initial number of cells, 144×10^6 per ml.

b U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

activity was affected only to a small degree. Considerably higher concentrations of the quaternary ammonium compound were required to cause a comparable bactericidal effect. A 50 ppm solution of QAC in hard water was insufficient to totally destroy all cells of the Salmonella suspension tested, while the same concentration in soft (distilled) water was more effective in a shorter period of time.

Similar results were observed for Escherichia coli, isolated from giblet cleaning machinery, and Pseudomonas aeruginosa, recovered from turkey wastes in the kill area of the plant. (Tables 4 and 5)

Activity of the QACs in hard water was markedly reduced against the gram-negative organisms, and they were generally the least effective of the germicides tested. They required higher concentrations and longer exposures than either the chlorine or iodine compounds in order to effect total destruction.

All four germicides tested were equally effective against Micrococcus luteus, which was isolated from the evisceration line of the turkey plant. The results in Table 6 suggest that this organism is destroyed most effectively by hypochlorites and iodophors. The pronounced inhibition of QAC by hard water salts evident when the gram-negative cultures were tested was not observed in this series of experiments, raising the possibility that the mechanism of activity of QACs may vary for different organisms.

Table 4. Destruction of Escherichia coli by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc.	Test water	pH of germicide solution	Average number of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
	ppm							
Iodophor A	6.0	distilled	3.62	0	0	0	0	0
	6.0	USDA[b]	7.30	91	0	0	0	0
Iodophor B	6.0	distilled	3.07	0	0	0	0	0
	6.0	USDA	6.70	TNC	0	0	0	0
QAC	50	distilled	6.24	0	0	0	0	0
	25		6.25	0	0	0	0	0
	12.5		6.27	TNC	52	58	3	3
	50	USDA	8.35	TNC	TNC	TNC	TNC	TNC
	25		8.43	TNC	TNC	TNC	TNC	TNC
	12.5		8.36	TNC	TNC	TNC	TNC	TNC
Hypochlorite	6.0	distilled	6.67	0	0	0	0	0
	6.0	USDA	8.48	0	0	0	0	0

a Initial number of cells, 79.6×10^6 per ml.

b U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Table 5. Destruction of Pseudomonas aeruginosa by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc.	Test water	pH of germicide solution	Average number of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
	ppm							
Iodophor A	6.0	distilled	3.78	0	0	0	0	0
	6.0	USDA[b]	7.70	3	0	0	0	0
Iodophor B	6.0	distilled	3.18	0	0	0	0	0
	6.0	USDA	6.67	12	0	0	0	0
QAC	100	distilled	5.89	0	0	0	0	0
	50		5.83	4	0	0	0	0
	100	USDA	8.81	TNC	TNC	TNC	223	74
	50		8.82	TNC	TNC	TNC	TNC	TNC
Hypochlorite	6.0	distilled	6.43	0	0	0	0	0
	6.0	USDA	8.72	37	0	0	0	0

a Initial number of cells, 26×10^6 per ml.

b U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Table 6. Destruction of Micrococcus luteus by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc.	Test water	pH of germicide solution	Average number of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
	ppm							
Iodophor A	6.0	distilled	3.54	TNC	1	0	0	0
	6.0	USDA[b]	7.64	43	0	0	0	0
Iodophor B	6.0	distilled	3.08	4	0	0	0	0
	6.0	USDA	6.67	103	0	0	0	0
QAC	50	distilled	6.20	4	0	0	0	0
	25		5.94	288	12	0	0	0
	50	USDA	8.65	42	3	0	0	0
	25		8.68	TNC	TNC	19	0	0
Hypochlorite	6.0	distilled	6.44	2	0	0	0	0
	6.0	USDA	8.73	43	2	0	0	0

a Initial number of cells, 13.5×10^6 per ml.

b U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

The most resistant vegetative culture tested was Streptococcus faecalis, isolated from a turkey neck on the evisceration line of the plant. (Table 7) Concentrations as high as 50 ppm were required to effect total destruction by the iodophors within one minute, while the hypochlorite remained totally effective in 15 seconds at 12.5 ppm. Iodophor activity against this organism appeared to depend more upon length of exposure than concentration, suggesting involvement of a time factor in the iodine reaching the site of inhibition. As with M. luteus, activity of the QAC against S. faecalis cells was not reduced by hard water, and may even have been potentiated. Fifty ppm QAC in distilled water destroyed all of the cells within 120 seconds, while only 30 seconds were required for the same concentration of QAC in hard water. Lower concentrations of QAC yielded a similar pattern.

Yeast of the genus Candida was isolated from a weighing scale in the turkey plant packing area following a cold water rinse. This organism was the only one tested which was resistant to hypochlorite, as shown in Table 8. Concentrations of 12.5 ppm required two minutes exposure in order to destroy all the cells in distilled water, and five minutes were necessary in hard water. Higher concentrations did not significantly increase the destruction rate in most cases.

Iodophors were effective in destroying all the yeast cells within 30 seconds at a concentration of 6 ppm in both soft and hard water.

Table 7. Destruction of Streptococcus faecalis by iodophor, hypochlorite and QAC germicides.

Germicide	Conc.	Test water	pH of germicide solution	Average number of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
Iodophor A	ppm 50	distilled	2.90	TNC	30	0	0	0
	25		3.17	TNC	TNC	23	0	0
	12.5		3.38	TNC	TNC	TNC	47	0
	50	USDA[b]	6.47	TNC	6	0	0	0
	25		6.69	TNC	11	0	0	0
	12.5		7.12	TNC	TNC	0	0	0
Iodophor B	50	distilled	2.30	41	0	0	0	0
	25		2.55	TNC	41	0	0	0
	12.5		2.80	TNC	TNC	0	0	0
	50	USDA	2.52	254	5	0	0	0
	25		3.02	TNC	95	0	0	0
	12.5		6.17	TNC	86	0	0	0
QAC	50	distilled	5.48	TNC	TNC	19	0	0
	25		5.57	TNC	TNC	TNC	89	0
	12.5		5.56	TNC	TNC	TNC	TNC	TNC
	50	USDA	8.15	18	0	0	0	0
	25		8.16	TNC	35	10	0	0
	12.5		8.25	TNC	TNC	TNC	14	48
Hypochlorite	12.5	distilled	8.11	0	0	0	0	0
	6.0		8.13	135	5	0	0	0
	12.5	USDA	8.23	0	0	0	0	0
	6.0		8.16	18	0	0	0	0

a Initial number of cells, 14.9×10^6 per ml.

b U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Table 8. Destruction of a yeast of the genus Candida by iodophor, hypochlorite, and QAC germicides.

Germicide	Conc.	Test water	pH of germicide solution	Average number of surviving organisms[a]				
				15 sec	30 sec	60 sec	120 sec	300 sec
	ppm							
Iodophor A	6.0	distilled	3.77	0	0	0	0	0
	6.0	USDA[b]	7.49	166	0	0	0	0
Iodophor B	6.0	distilled	3.23	4	0	0	0	0
	6.0	USDA	6.81	40	0	0	0	0
QAC	50	distilled	6.36	4	0	0	0	0
	25		6.32	83	27	0	0	0
	12.5		6.32	TNC	223	2	0	0
	50	USDA	8.60	TNC	168	0	0	0
	25		8.68	TNC	TNC	63	5	1
	12.5		8.61	TNC	TNC	TNC	TNC	135
Hypochlorite	50	distilled	8.35	TNC	TNC	40	0	0
	25		7.92	TNC	TNC	30	0	0
	12.5		7.13	TNC	TNC	122	0	0
	50	USDA	8.73	TNC	107	0	0	0
	25		8.64	TNC	TNC	112	2	0
	12.5		8.58	TNC	TNC	TNC	47	0

a Initial number of cells, 9.6×10^5 per ml.
b U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

A small degree of inhibition was evident when these compounds were used in hard water, but not enough to effect germicidal activity in practical use situations.

The QAC compound was active against yeast when concentrations of 50 ppm were used in soft water. Lower concentrations, particularly in hard water, were not as effective.

Bacillus licheniformis was isolated from a saw used to split carcasses in half on the kill floor of the beef processing plant. A spore suspension of this organism was tested against all four germicides at the highest concentration considered practical for everyday use in meat processing plants, 200 ppm, and exposure time was extended to a maximum of twenty minutes. Table 9 reflects the high resistance of the spores to all the germicides tested.

The iodophors and QAC were completely ineffective against the spores, while the hypochlorite was partially able to reduce the total number after twenty minutes exposure time. It was noted that greater reduction of spores occurred when the hypochlorite was diluted with hard water and the pH was slightly lowered from 8.97 to 8.87. Another series of experiments were performed in which the hypochlorite diluent was 0.25 M phosphate buffer, and the pH of the germicide solution was 7.1. Under these conditions, 200 ppm hypochlorite totally destroyed all spores in the suspension within 2 minutes, effecting a greater than ten-fold increase in destruction rate.

Table 9. Destruction of Bacillus licheniformis spores by iodophor, hypochlorite and QAC germicides.

Germicides	Conc.	Test water	pH of germicide solution	Average percent of surviving spores[a]				
				30 sec	2 min	5 min	10 min	20 min
	ppm							
Iodophor A	200	distilled	2.43	100	100	100	100	100
	200	USDA[b]	2.65	100	100	100	100	100
Iodophor B	200	distilled	1.97	100	100	100	100	100
	200	USDA	2.01	100	100	100	100	100
QAC	200	distilled	6.57	100	100	100	100	100
	200	USDA	8.47	100	100	100	100	100
Hypochlorite	200	distilled	8.97	100	100	100	100	64.5
	200	USDA	8.87	100	100	100	100	11.9
	200	Buffer (pH 7.0)	7.10	100	0	0	0	0

a Initial number of spores, 1.17×10^4 per ml.

b U. S. Department of Agriculture, 500 ppm ($CaCO_3$).

Range of Germicidal Activity

The spectrum of activity on the vegetative cells tested varied for each germicide. As shown in Figure 1, 12.5 ppm hypochlorite destroyed all bacterial suspensions tested within 15 seconds in both distilled and hard water. Yeasts were resistant to very dilute hypochlorites, and concentrations as high as 50 ppm were unable to appreciably increase the rate of destruction.

Hypochlorites were the only germicide tested which exhibited any sporicidal activity at a concentration of 200 ppm, which is the maximum level acceptable for most practical applications. The effectiveness of these compounds in killing spores varies greatly with the pH of the solution (Figure 2). A reduction in pH of 0.1 unit caused a greater than 50 percent decrease in the survival rate of spores after 20 minutes exposure. When the pH was buffered down to 7.1, the hypochlorite solution destroyed all spores within 2 minutes.

Figures 3 and 4 indicate the wide range of bactericidal activity of iodophors at low concentrations. The acidity of iodophor A was insufficient to maintain optimum pH levels in high dilutions, yet both compounds destroyed all vegetative cells except Streptococcus faecalis within 15 seconds. Of the non-sporeforming bacteria tested, the streptococci were the most resistant to all the germicides studied.

Neither the hypochlorite nor iodophor germicides tested were

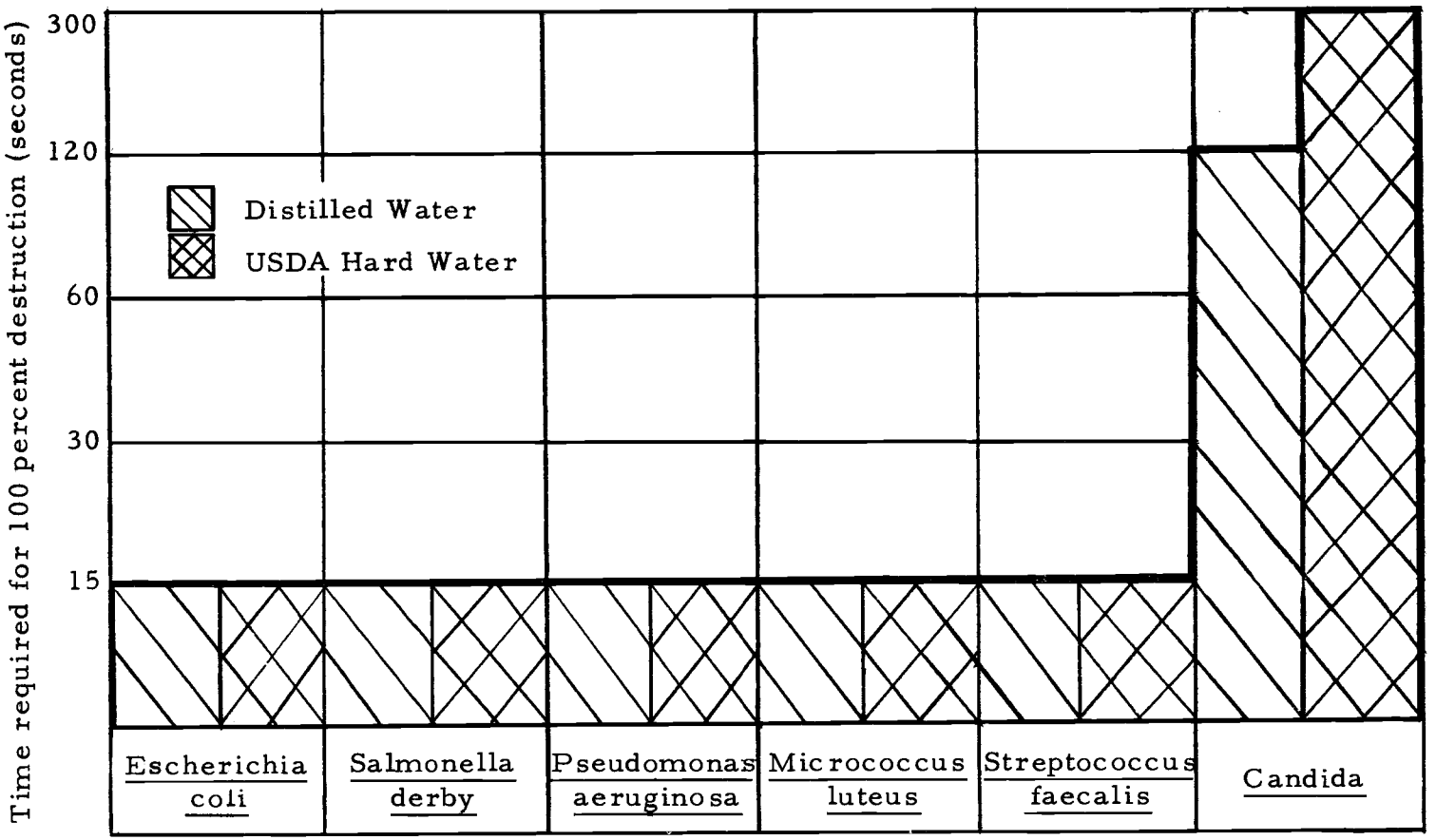

Figure 1. Relative destruction rates of vegetative cells by 12.5 ppm hypochlorite solutions in distilled and USDA hard water.

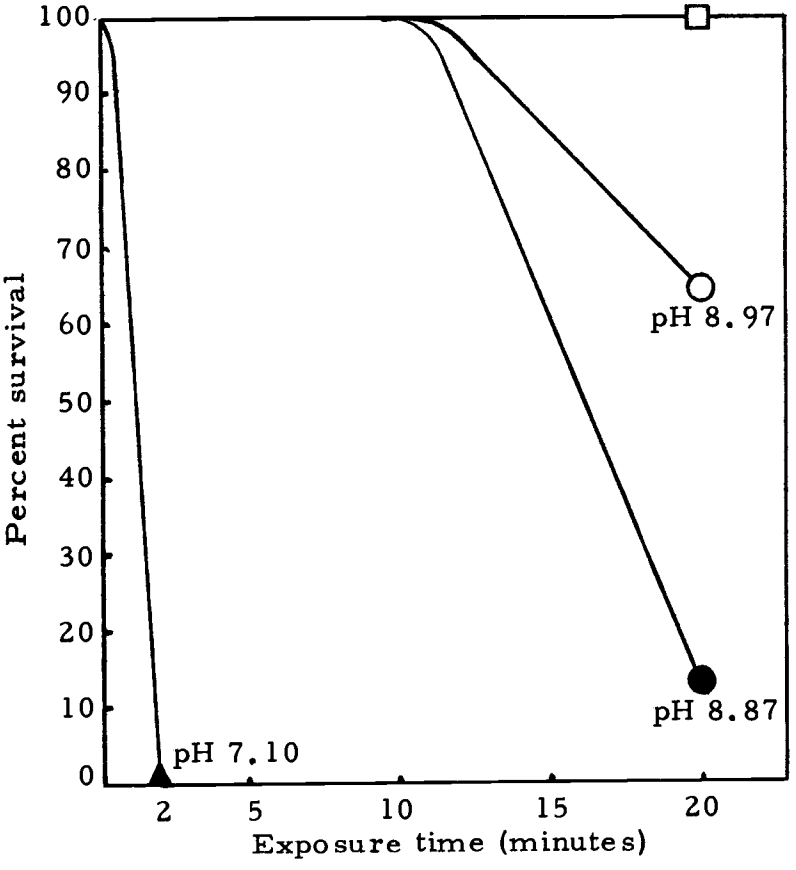

Figure 2. Rate of destruction of Bacillus licheniformis spores by 200 ppm iodophor, QAC and hypochlorite germicides. Symbols: □ iodophor A and B, QAC; ○ hypochlorite (distilled); ● hypochlorite (USDA); ▲ hypochlorite (buffered).

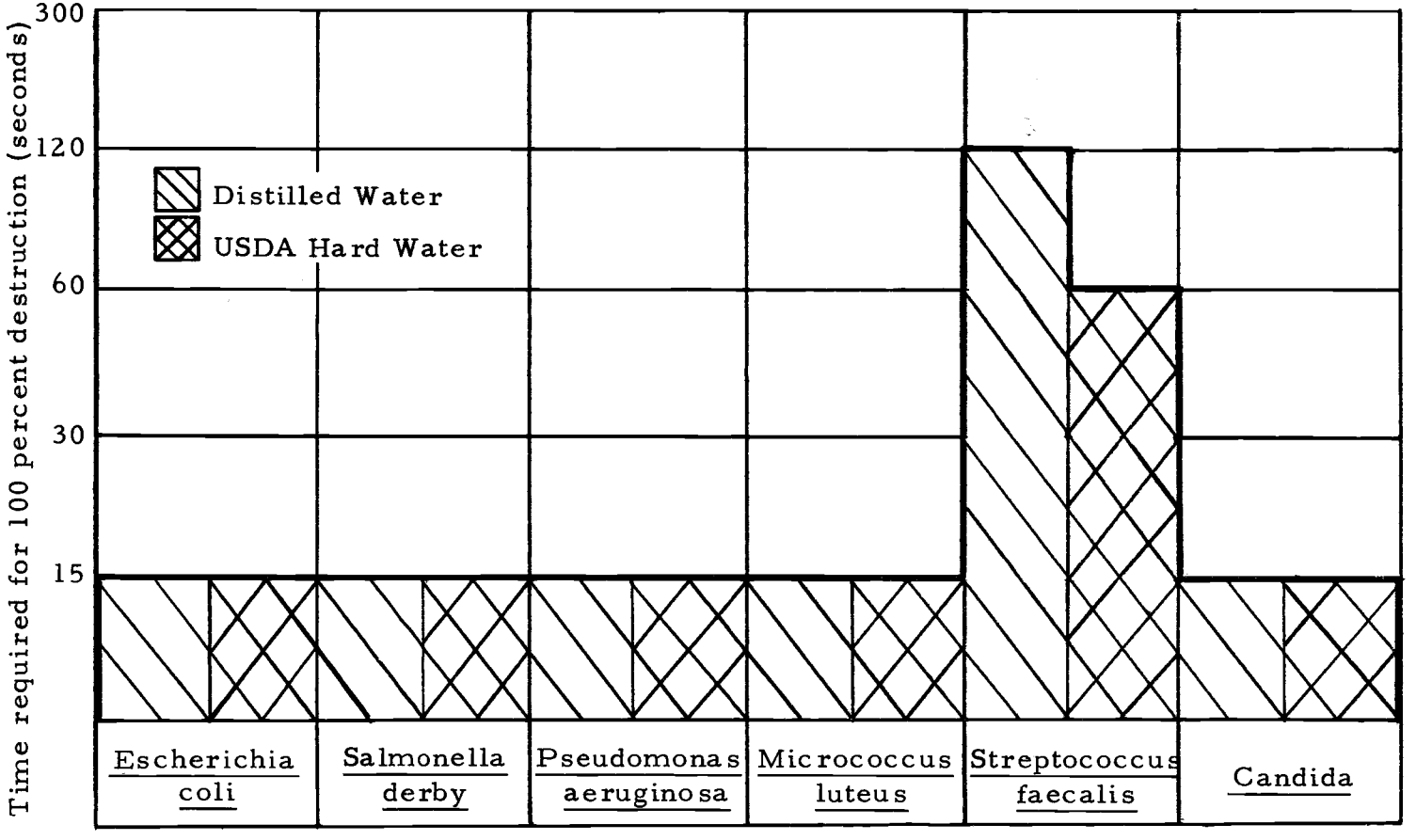

Figure 3. Relative destruction rates of vegetative cells by 12.5 ppm iodophor A solutions in distilled and USDA hard water.

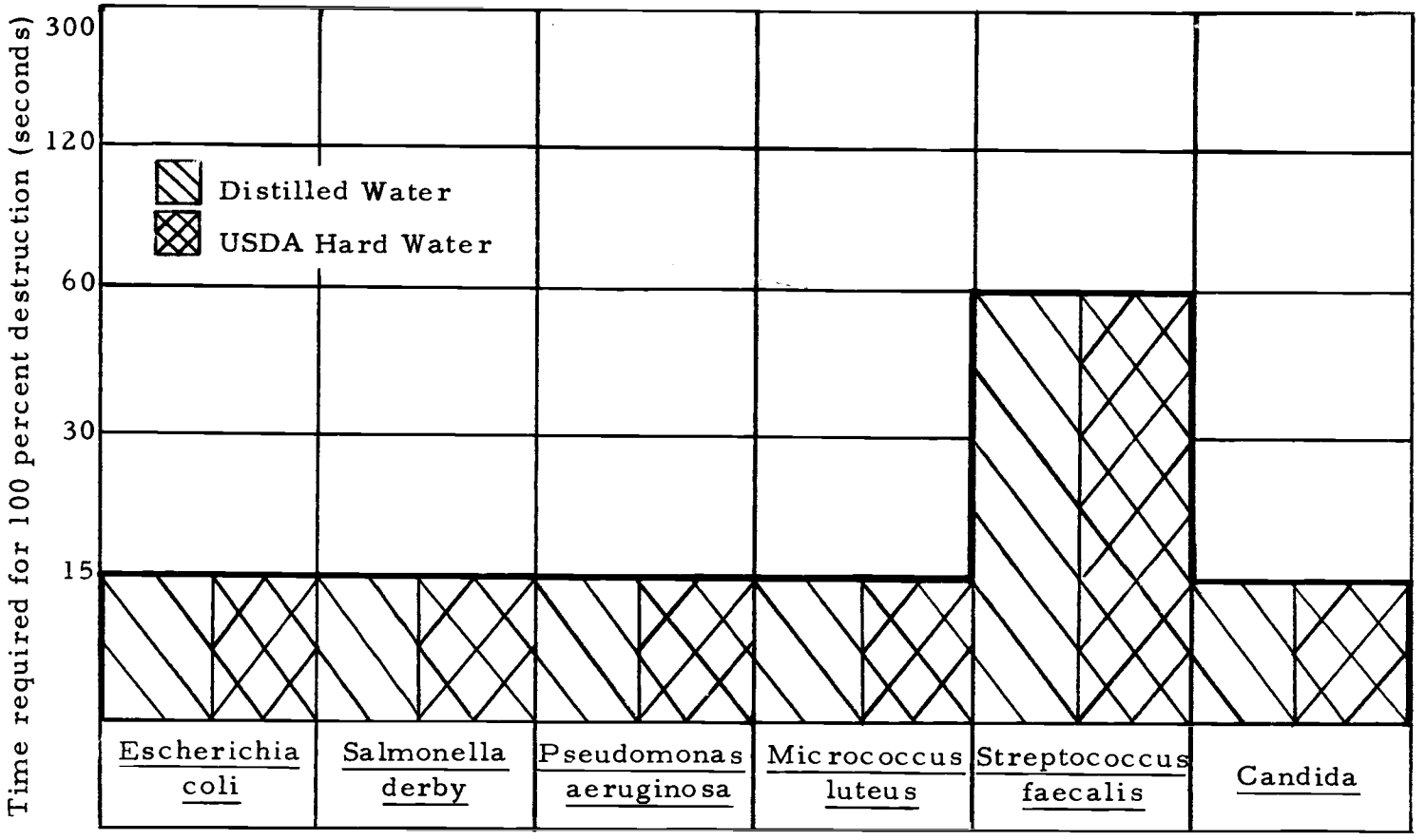

Figure 4. Relative destruction rates of vegetative cells by 12.5 ppm iodophor B solutions in distilled and USDA hard water.

markedly affected by water hardness in the range of approximately 0 to 500 ppm $CaCO_3$ at low concentrations.

The general susceptibility of the quaternary ammonium compounds to hard water is reflected in Figure 5. Higher concentrations and longer exposure periods were required for the QAC to approximate the germicidal activity of the other compounds tested. The gram-negative cultures were all at least partially resistant to relatively high concentrations in hard water for the maximum exposure period of 5 minutes. The QAC tested was ineffective against spores.

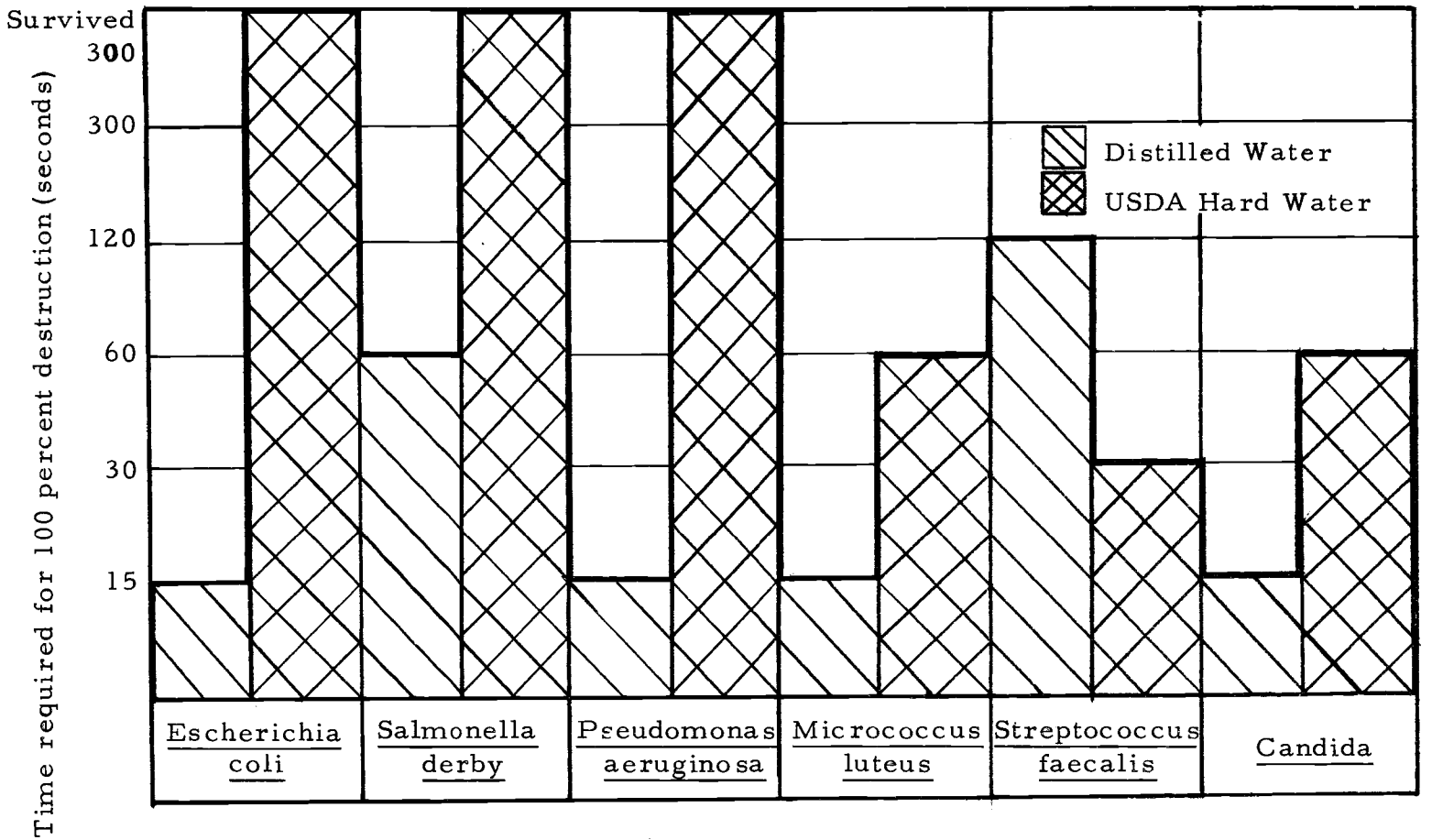

Figure 5. Relative destruction rates of vegetative cells by 50 ppm quaternary ammonium solutions in distilled and USDA hard water.

DISCUSSION

The type of organisms isolated from meat and poultry plants were similar to those recovered by other investigators in typical processing plants. The Pseudomonas - Achromobacter group predominated in areas where low temperature handling had reduced the numbers of competitive bacteria to relatively low levels. The majority of organisms found in this study were recovered from surfaces which, at one time or another during processing, came into direct contact with the final product. The high level of contamination on both processing surfaces and the final products indicate that survival of these organisms under conditions which are designed to destroy or reduce them are more or less assured. Mishandling and delays in distribution systems serve to provide further opportunities for these organisms to flourish, and make an expensive commodity unattractive if not unsalable or unsafe to the consumer.

Each of the organisms tested in these studies were freshly obtained from their natural environment, and represent a spoilage, indicator, or pathogenic contaminant common to fresh beef or poultry. The germicides used to destroy these organisms were commercial products available in the market and applied in a wide variety of food processing and dairy operations.

Of the three general types of germicides studied, the results

indicate that the quaternary ammonium compound (QAC) was the least suitable for food processing environments. It was slower acting and required higher concentrations than either of the halogen compounds. In the presence of 500 ppm USDA hard water (calculated as $CaCO_3$), the QAC was ineffective against the gram-negative bacteria chiefly responsible for meat spoilage, Pseudomonas. Salmonella, an important pathogen frequently associated with poultry, was also highly resistant to the QAC tested in the presence of hard water, as was Escherichia coli. As a sporicide, the QAC tested was totally ineffective.

The reported bacteriostatic activity of these compounds is of limited value in the food processing plant, where germicidal residues on equipment are short-lived, and undesirable on the final product.

The bactericidal activity of hypochlorite and iodophor compounds tested in this study was quite similar. Both types of compound showed a high rate of destruction in both soft and hard water in low concentrations. At a level of 12.5 ppm, a concentration too dilute to overcome the buffering effect of the alkaline hard water, both the high and low acid iodophors exhibited identical destructive rates on vegetative cells. Only Streptococcus faecalis cells survived these concentrations for more than 15 seconds, and that organism represented the most resistant bacterial culture for all the germicides tested. The iodophors were notably ineffective against spores despite

concentrations of 200 ppm and exposures of 20 minutes. In the case of iodophor B, the pH of the germicidal solution was below 2.0, yet no reduction of spores was observed.

At a concentration of 12.5 ppm, the hypochlorite killed all the bacterial cultures tested within 15 seconds, including the relatively resistant S. faecalis cells. At lower concentrations, the hypochlorite exhibited the most rapid rate of destruction of any of the germicides.

In distilled water, the average pH of a 12.5 ppm solution of hypochlorite was 7.7, and in USDA hard water the pH was increased to 8.8. The higher alkalinity did not affect the rate of destruction of bacterial cultures but measurably increased the time required to kill yeast. When higher concentrations were applied to spore suspensions, the effect of pH became more pronounced. A concentration of 200 ppm hypochlorite in hard water had a pH slightly lower than a similar solution in soft water, and a significant increase in sporicidal activity was noted. The use of pH 7.0 buffer as germicide diluent caused a greater than 10-fold increase in the spore destruction rate, and no surviving spores were detected. Other workers have reported similar results for lower concentrations of hypochlorites on vegetative cells, and have demonstrated the stability of these germicides even when acidified to pH 4.0 (40).

Excess alkalinity of hypochlorite solutions may serve to neutralize or even reverse the effect of higher concentrations in

practical applications. Water hardness and the presence of organic matter also affect the effectiveness of hypochlorite under practical conditions.

In this study excess organic matter was carefully excluded from the reaction flasks in order to compare the activity of each germicide under controlled conditions of water hardness and concentration. While it may be argued that such conditions do not represent those found in practical applications, it is also important to note that surfaces bearing organic matter will not be effectively sanitized by any chemical agent unless the particulate matter is first removed. This study has indicated that the majority of microbial contaminants on equipment surfaces are associated with such matter.

In view of the bactericidal efficiency of both hypochlorite and iodophor compounds in low concentrations, either type of germicide appears to be well suited to a wide range of applications in the food processing industry. Hays, Elliker and Sandine (41) in a study similar to this work, reported that the combined action of hypochlorite followed by iodophor treatment served to apply the advantages of both germicides, effecting practically complete destruction of the organisms tested. Shorter applications of higher dilutions of both types of germicide may be economically comparable to more extensive use of either one, and would almost certainly result in a higher level of sanitation.

SUMMARY

Microbial contaminants were isolated from beef and poultry processing plants by conventional contact plate and swab sampling techniques. Generally high levels of contamination were found, particularly where residues of organic matter were permitted to accumulate.

Isolates representing spoilage, indicator, and pathogenic contaminants common to fresh beef and poultry were selected and subjected to various concentrations of hypochlorite, iodophor, and quaternary ammonium compounds in both distilled and USDA hard water (500 ppm $CaCO_3$). Vegetative cells were exposed for intervals of from 15 to 300 seconds, and spore suspensions were treated for up to 20 minutes.

The results of the studies indicated that the QAC tested was highly selective, particularly when diluted with hard water. Gram-negative organisms exhibited a marked resistance to this compound under most conditions, and higher concentrations for longer exposure periods were required to even approximate the bactericidal activity of the other germicides tested. The QAC exhibited no sporicidal activity at a level of 200 ppm.

Both low and high acid iodophor formulations were tested and found to be equally effective in destroying a wide range of vegetative

cells in a very short time at low concentrations. The degree of water hardness did not appreciably affect their germicidal activity. Even with relatively resistant organisms, such as S. faecalis, destruction with 12.5 ppm solutions appeared to be more a function of time than of pH. Higher concentrations did not significantly increase the destruction rate. The iodophors were equally effective in killing yeast, but exhibited no sporicidal activity at concentrations of 200 ppm.

The hypochlorite and iodophor germicides had very similar destruction rates at any given concentration. The hypochlorites were distinctly less active in killing yeast suspensions, possibly due to the effect of the greater cell mass imparting a higher chlorine demand. The hypochlorites were the only germicide tested which were sporicidal at concentrations of 200 ppm. When the pH of these solutions was buffered to pH 7.1, a ten-fold increase in the rate of sporicidal activity was observed.

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