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Incidence and types of bacteria associated with giblets of commercially processed turkeys

Richard Harry Salzer
Iowa State University

INCIDENCE AND TYPES OF BACTERIA ASSOCIATED WITH GIBLETS OF COMMERCIALLY PROCESSED TURKEYS

by

Richard Harry Salzer

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Signature was redacted for privacy.

Head of Major Department

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Dean of Graduate College

Iowa State University
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I. INTRODUCTION

During the past 35 years the number of turkeys produced for table use has increased about five-fold to an estimated 98.7 million turkeys in 1964 with California, Minnesota and Iowa being the leading three states in production. Rapid expansion of the turkey industry did not occur, though, until after World War II. This increase in production was due in part to increased feed efficiency, improved strains and new breeds of birds, mechanical handling of birds and feeds, new drugs for disease control, and improved processing techniques. Although freezing has made turkeys available all year, peak demand still occurs in November and December, with the largest marketing during the second week before Thanksgiving. The slaughtering of birds follows the demand with most processing beginning in late June or early July and stopping by January 1. If interest in further processed items such as rolls, roasts, steaks, and other products continues, the slaughtering season may start earlier in the year.

Good sanitation and washing play important roles in the preparation of these 99 million birds for market. Studies on chicken processing have shown that birds prepared under clean, sanitary conditions had a shelf life from 3 to 7 days longer than those processed under insanitary conditions. When properly done, washing procedures result in the removal of

dirt, debris, and a large portion of the surface bacterial population. That turkey producers have had a continuing interest in selling a high quality product can be noted from the fact that in 1955, four years before mandatory inspection of poultry processed for interstate shipment was required, 50 percent of the turkeys marketed were certified under U. S. Department of Agriculture inspection. In 1959, the percentage of turkeys inspected increased from 72 to 79 percent while the percentage of chickens inspected rose from 26 to 83 percent. In 1963, 84.2 percent of all turkeys marketed were federally inspected.

With the expansion of turkey processing, turkey giblets have become increasingly important to the processor when sold either as part of the whole bird or for their value if sold separately, such as in European markets. Giblets comprise about 4.5 - 6.5 percent of the chilled ready-to-cook weight of Broad Breasted Bronze and Beltsville turkeys (Sweet et al., 1954). The heart and gizzard composed 3 percent of the total cooked meat (Miller, 1964). Giblets represent about 10 percent of the total value of the eviscerated turkey.

During processing, the giblets (gizzard, heart and liver) are removed from the bird being prepared. The inner lining of the gizzard is removed and all the giblets are washed to remove blood and debris which may have contaminated the giblet

surface during evisceration. In some cases the washed giblets are placed in 30 pound cans and frozen. These frozen giblets are then used in processed foods such as strained turkey for babies. In most instances, one gizzard, one heart and one liver are placed in a parchment pouch and stuffed into the neck cavity of whole birds prepared for freezing. In packaging, it is very unlikely that all the giblets removed from one turkey will be placed in the same pouch or even that one of the giblets will be placed in the same bird from which it was removed.

Since the giblets are processed separately from the turkey carcass, it is conceivable that the microbiology of these edible viscera could be different from that of the whole turkey. Several investigators have studied the microbiology of poultry meat and the effect of processing on the microorganisms of the flesh. Other workers have been concerned with the incidence of Salmonella on giblets during processing and in retail markets, but most of the work reported did not include total microbial load on the giblets or the presence of pathogenic bacteria other than Salmonella or indicators of fecal pollution. The surface structure of the giblets is not identical and it is possible that during frozen storage microorganisms could survive longer on the surface of one kind of giblet than on another. Based on length of survival, one type of giblet could then be selected for determinations of micro-

biological quality. Information concerning populations of microorganisms on giblets is needed.

The purpose of this study was to determine the bacterial quality of turkey giblets as they are usually processed, to discern what, if any, role these giblets may have in the transmission of food-borne disease to man, to investigate the bacterial quality of giblets after they were held in frozen storage for specific periods of time, and to isolate and identify the microorganisms on the giblet surface so that improved control steps might be taken if needed.

II. LITERATURE REVIEW

A. Incidence and Types of Microorganisms Generally Associated with Processed Poultry

1. Incidence of microorganisms

It is well accepted that the microorganisms that cause the spoilage of flesh foods come from the animal being slaughtered or from the environment in the processing plant, such as workmen and their cutting tools, water used in preparing the food, or contaminated air. The following are responsible for the bacterial contamination of processed poultry: flora on the skin of the live bird, mud and filth from the feet and crop material, feces forced from the bird during processing, water supplies, slush ice, and equipment (Gunderson et al., 1946; Goresline et al., 1951; Walker and Ayres, 1956; Barnes, 1960a). Almost all of the contaminating organisms on an eviscerated bird, both initially and after storage, are found on the skin, the surface of the visceral cavity, and cut surfaces (Lochhead and Landerkin, 1935; Gunderson et al., 1947; Ayres et al., 1950; Barnes, 1960b).

The average number of bacteria on chilled chickens washed mechanically before evisceration was recorded by Goresline et al. (1951) to be 28,000 per $cm.^2$ of skin surface, while the average population of bacteria on eviscerated birds washed and ready to pack was 17,000 per $cm.^2$. Gunderson et al. (1954)

noted that the average viable bacterial count of birds arriving on the evisceration line was 26,400 per cm.^{2}; bacterial numbers were reduced to 3,460 per cm.^{2} on completion of evisceration. However, Drewniak et al. (1954) observed that the count increased from 9,500 per cm.^{2} after vent cutting and before evisceration to 18,000 per cm.^{2} after evisceration and inspection. Walker and Ayres (1956) reported that the skin of the live bird had a load of approximately 1,500 per cm.^{2} and the final product had a count of approximately 35,000 per cm.^{2} of skin surface. According to May (1962), the mean initial number of bacteria on uncut chickens was 2,100 bacteria per cm.^{2}; bacterial populations increased approximately six-fold during cutting and packaging. May (1961) discovered that the transfer station for moving chicken carcasses from the picking to the evisceration line was the most consistent source of large increases in bacterial numbers. He believed that this resulted from lack of hand-washing facilities at this station.

Goresline et al. (1951) recovered 6,500 bacteria per cm.^{2} from the visceral cavity while samples taken from the skin of the same birds had counts of 40,000 per cm.^{2} on the breast, 30,000 per cm.^{2} on the thigh, and 30,000 per cm.^{2} on the back. Later studies by Walker and Ayres (1956, 1959) indicated that, in general, the bacterial loads from the visceral cavity of processed chickens and turkeys were lower than those obtained

from the skin.

A report on the number of bacteria recovered from frozen packaged livers and gizzards of chickens was made by Gunderson et al. (1954). Total viable bacterial counts ranged from 900 to 1,134,000 per g. of liver tissue and from 8,600 to more than 654,000 per g. of gizzard tissue. Essary and Howes (1960) observed that only 0-2 organisms were recovered from chicken liver tissue when the surface of the bird was disinfected with 70 percent ethyl alcohol before opening. The bacteria associated with giblets of commercially processed turkeys have been discussed by Salzer et al. (1964). There are several reports in the literature on the incidence of salmonellas recovered from the giblets of chickens and equipment used to process the giblets (Cherry et al., 1943; Felsenfeld et al., 1948; Galton et al., 1955; Brobst et al., 1958; Morris and Ayres, 1960). Other workers have reported on Salmonellae isolated from turkey giblets, processing equipment and personnel on the eviscerating line (Edwards et al., 1948; Browne, 1949; Felsenfeld et al., 1950; Tailyour and Avery, 1960; Wilson et al., 1961; Sadler et al., 1961).

2. Types of microorganisms

Any of a number of microbial species are likely to be recovered from chicken meat immediately after the bird has been killed and processed. Gunderson et al. (1947) found that

the following genera and types of microorganisms were recovered from the surface of freshly killed chickens: Escherichia, Aerobacter, Micrococcus, Alcaligenes, Achromobacter, Paracolobactrum, Flavobacterium, Proteus, Bacillus, Pseudomonas, Staphylococcus, Microbacterium, Eberthella (Salmonella), Sarcina, diptheroids, Gaffkya, Streptococcus, Corynebacterium gallinarium, Neisseria, Oidium, and Torula. Representatives of the following genera were isolated by Ayres et al. (1950) from fresh and defrosted, frozen cut-up chickens: Pseudomonas, Micrococcus, Achromobacter, Flavobacterium, Alcaligenes, Proteus, Bacillus, Sarcina, Streptococcus, Eberthella, Salmonella, Escherichia, Aerobacter, Streptomyces, Penicillium, Oospora, Cryptococcus and Rhodotorula. The predominant types of organisms on fresh poultry were also reported by Stadelman et al. (1957) to be Pseudomonas, Flavobacterium, and Saccharomyces. Barnes and Shrimpton (1958) listed the following psychrophilic bacteria on fresh chilled eviscerated chickens:

> About 25 percent pigmented Pseudomonas spp.
>
> About 60 percent Achromobacter spp.
>
> About 15 percent miscellaneous types including Cytophaga spp. and Flavobacterium spp.

Of 37 organisms isolated from fresh broilers, Thatcher and Loit (1961) identified 13 Pseudomonas spp.; six Alcaligenes spp.; five Flavobacterium spp.; five Aerobacter spp.; four Achromobacter spp.; and four Escherichia spp.

Of the types of organisms recovered from chickens entering a processing plant, Barnes (1960a,b) noted that Achromobacter spp. which grew at low temperatures predominated on the feet, feathers and bodies of chickens, with some Corynebacterium spp. present. She learned that following evisceration and washing Pseudomonas formed a high proportion of the total population.

B. Effect of Washing on Reducing Bacterial Numbers

1. Poultry meat

The effect of fresh flowing water in reducing the bacterial population on surfaces of poultry carcasses has been demonstrated by the following investigations. Washing birds after they were eviscerated was found by Gunderson et al. (1946) to reduce the bacterial count but not to eliminate the coliforms. Drewniak et al. (1954) determined that the bacterial count per $cm.^2$ of chicken skin doubled after evisceration and that the most contaminated pieces of equipment were those which did not use running water and those used for washing carcasses. It was reported by Wilkerson et al. (1961) that a final spray rinsing reduced the total aerobic counts on turkey carcasses. With chickens, final washing by a spray washer after evisceration reduced total counts significantly (May, 1961). It was observed by Shrimpton and Stevens (1961)

that chickens cooled in mechanical chillers had a slightly lower count than those in static slush ice. Kotula et al. (1962) stated that a significant decrease in skin bacteria occurred when chickens were chilled in a continuous counter-flow tumbler chiller. This was probably due to the fact that water overflowed continuously from the chiller, resulting in dilution and removal of bacteria by the overflow.

2. Equipment

The importance of running water on keeping the bacterial contamination low on processing equipment has been shown by others. Goresline et al. (1951) found that conveyer pans used to hold the viscera during veterinary inspection had a count of only 9,500 bacteria per cm.^{2} even though there was frequent fecal contamination. These workers believed that the counts were low because the pans were washed thoroughly with pressure sprays on the return trip. Only one isolation of Salmonella was made by Galton et al. (1955) from an unspecified number of samples from trays or tables containing edible viscera where the evisceration table was equipped with running water. In contrast, 50 isolations were made from plants in which the edible viscera were held in pans and the water was changed only when the pans were filled. According to Drewniak et al. (1954), the use of water could minimize the possibility of recontamination of the carcass by washing away bacteria from

equipment surfaces, tabletops, and hands of workers. Wetzler et al. (1962) obtained bacterial, yeast, and mold counts from unspecified food contact surfaces before and after usual maintenance practices in two large poultry processing plants. The last mentioned workers reported that, in general, good clean-up resulted in a marked lessening of coliforms and other Gram negative organisms. The residual flora after cleaning was largely composed of fecal streptococci, staphylococci, and yeasts. The cocci and yeasts were probably more resistant to the cleaning compounds used.

C. Importance of Poultry in the Transmission of Salmonellosis and Staphylococcal Food Poisoning

1. Reports of poultry being associated with food poisoning outbreaks

Reports of milk-borne disease outbreaks were begun in 1923 (Dauer, 1961) but it was not until 1938 that the U. S. Public Health Service began to collect annual reports of outbreaks of food-borne diseases and of epidemics caused by faulty sanitation. Before 1952, staphylococcal food poisoning in man had not been traced to avian origin, although the species and types of staphylococci recoverable from fowl had enterotoxic potentialities. The staphylococci are not common causes of disease among birds and their contamination of poultry eggs and poultry meat may be derived from non-avian

sources (Brandly, 1951). Outbreaks of food poisoning were recorded but there was no breakdown as to causative agent or type of food involved. There were some reports in the literature of food poisonings associated with poultry prior to 1951, but these investigators did not describe the causative agent (Geiger, 1923; Frank, 1940; Fuchs, 1941). Outbreaks in the United States of salmonellosis and staphylococcal food poisonings associated with poultry for the years 1956-1960 are presented in Table 1.

Table 1. Incidence of salmonellosis and staphylococcal food poisonings associated with poultry during 1956-1960

Year	Salmonellosis		Staphylococcal food poisoning	
	Outbreaks	Cases	Outbreaks	Cases
1956[a]	4			
1957[b]	6	462	10	340
1958[c]	8	162	6	214
1959[c]	5	109	7	1050
1960[d]	4	350	8	682

[a]Source: Dauer and Sylvester, 1957.

[b]Source: Dauer, 1958.

[c]Source: Dauer and Davids, 1959, 1960.

[d]Source: Dauer, 1961.

The importance of poultry in relationship to other foods as a source of food poisoning may be seen in several reports. Over 30 percent of the cases of food-borne diseases reported by the states to the National Office of Vital Statistics were associated with poultry and poultry dishes (Atkinson, 1957). Dack (1962) recorded that turkeys accounted for 14 and chickens for nine of 137 outbreaks of staphylococcal food poisoning during the years 1956-1961. In a summary of food-borne illness in the United States from 1957 to 1962, poultry was reported to be responsible for 28 of 206 outbreaks (13.6 percent) and 1563 of 7868 cases (20 percent) of food poisoning caused by staphylococcus toxin; salmonellosis from poultry accounted for 16 of 69 outbreaks (23.2 percent) resulting in 1633 cases (35.1 percent of the total number of cases) (Dack, 1963). The extent of contamination of poultry with Salmonellae is presented in detail in the following section.

2. Incidence and species of Salmonella recovered from poultry

According to many investigators, poultry constitutes one of the largest reservoirs of Salmonellae and one of the most important sources of human salmonellosis (Edwards et al., 1948; Goresline, 1948; Hinshaw and McNeil, 1948; Hinshaw, 1949; Felsenfeld, 1951; Quist, 1962; Kampelmacher, 1963). Several

studies show recoveries of Salmonellae from poultry during processing. Galton et al. (1955) visited three Florida chicken processing plants and recovered salmonellas in the following proportions: 86 (16.6 percent) of 517 samples from one plant, 97 (19.9 percent) of 486 samples from the second plant, and 13 (5.3 percent) of 241 samples from the third plant visited. Morris and Ayres (1960) recovered Salmonella spp. from only 12 of 2500 samples taken in a federally inspected turkey processing plant. In Canada, Thatcher and Loit (1961) isolated salmonellas from 14 of 100 broilers examined. In Great Britain, Dixon and Pooley (1962) visited two turkey processing plants and isolated salmonellas from 36 (19.3 percent) of 187 specimens from one plant and 10 (7.8 percent) of 129 specimens from the second plant. With the exception of two isolations of S. menston and one strain of S. stanley, all of the salmonellas isolated were S. typhimurium.

The following reports indicated attempts to isolate salmonellas from poultry viscera and evisceration equipment. The incidence of multiple serotype isolations of salmonellas from turkey livers was studied by Edwards et al. (1948) who recovered two or more species of Salmonella in each of 23 turkey livers, 15 turkey intestines, and 11 sample of intestine and liver. Browne (1949) isolated S. typhimurium from the following sources in a turkey processing plant (Table 2):

Table 2. Isolations of S. typhimurium from a turkey processing plant

Source	Total number of samples	Number positive	Number negative
Livers (with lesions)	4	2	2
Viscera trays at the end of processing (each sample is a pool of six trays)	9	9	0
Viscera trays at the beginning of afternoon processing (each sample is a pool of six trays)	1	1	0
Pans for cleaned giblets	2	1	1
Hands of eviscerators, trimmers and inspector	5	5	0

In three chicken processing plants, the highest percentages of samples positive for Salmonella were obtained from the edible viscera and from the table on which edible viscera were wrapped, with an average of 31 percent positive (Galton et al., 1955). In other studies (Brobst et al., 1958; Morris and Ayres, 1960), however, no salmonellas were isolated from the eviscerating table or from the drainage of giblets. Tailyour and Avery (1960) isolated salmonellas from the viscera and intestinal contents of only four (0.76 percent) of 523 market weight turkeys. All four isolates were S. derby. Sadler et al. (1961) isolated Salmonella species from 2.4 percent of the

intestinal or cecal samples of turkeys on the processing line.

Studies have been made of the occurrence of salmonellas in market poultry livers. Cherry et al. (1943) found no Salmonellae in three samples of chicken livers procured from retail markets in the Lexington, Kentucky area. Felsenfeld et al. (1948) took samples of liver, leg muscle, and mesenteric glands from 50 apparently healthy chickens raised in Puerto Rico and sold in approved and inspected commercial establishments. Seven of these samples (14 percent) harbored Salmonellae. Felsenfeld et al. (1950) found that eight (8.7 percent) of 91 uninspected livers sampled in retail shops and 16 (14.9 percent) of 107 uninspected liver samples collected in homes were positive for salmonellas. In another market study, Wilson et al. (1961) found 22 (24 percent) of 93 chicken giblets contaminated with salmonellas and also isolated salmonellas from two turkey gizzards.

The following reports indicate the recovery of salmonellas from infected poultry. In the United States, Edwards (1939) reported that of 223 cultures of Salmonellae derived from 100 outbreaks of disease in fowls, S. typhimurium was found more frequently than all the other types combined. He recovered the following species from 31 outbreaks in turkeys: S. typhimurium (14 outbreaks), S. derby (4), S. anatum (2), S. senftenberg (2), S. bareilly (2), S. bredeney (2), S. newington (1), S. newport (1), S. worthington (1), and S. minne-

sota (1). Hinshaw et al. (1944) noted that a total of 47 Salmonella types other than S. pullorum and S. gallinarum were isolated from poultry and 41 of these were also isolated from man. Later Edwards et al. (1948) recovered S. pullorum in 50 percent of the isolations from chickens and S. typhimurium in approximately 22 percent. Taylor (1960) summarized the number and types of Salmonellae isolated from chickens and turkeys in England and Wales during the years 1956 through 1958. The following types were isolated from chickens: S. africana, S. amager, S. california, S. cholerae-suis, S. meleagridis, S. muenchen, S. senftenberg, S. thompson, S. typhimurium. From turkeys, the following species were recovered: S. anatum, S. binza, S. breaenderup, S. cubana, S. infantis, S. minneapolis, S. montevideo, S. new-haw, S. orion, S. stanleyville, S. thompson, S. typhimurium, S. worthington. Bigland (1963) noted that only 15 of 1242 Salmonellae isolations in 12 years came from birds other than turkeys or chickens. Excluding S. gallinarum and S. pullorum, he found the following types to be present: S. typhimurium (28 percent), S. thompson (19 percent), S. heidelberg (17 percent), S. bareilly (10 percent), and S. oranienburg (8 percent). Infection of poultry in most countries has been reported to be mainly with S. typhimurium, S. thompson, S. anatum, and S. bareilly (Kampelmacher, 1963).

3. Correlation of coagulase test and enterotoxin production

The determination of the types of staphylococci responsible for enterotoxin production is described in this section. It was learned by Feldman (1946) that 23 of 27 strains of staphylococci which produced enterotoxin were classified as Staphylococcus aureus and four as S. albus. Of the S. aureus strains studied, he noted that 78 percent were coagulase positive, 61 percent were hemolytic, and 14 percent fermented mannitol; the S. albus group had none of these characteristics. Hussemann and Tanner (1949) ascertained that, of 28 strains of staphylococci from foods incriminated in food poisoning outbreaks, 96.4 percent were coagulase positive. In a study by Evans and Niven (1950) of 22 known enterotoxin producing staphylococci and 92 other cultures of staphylococci, it was discovered that all toxigenic strains were coagulase positive and appeared to be identical with all the other coagulase positive strains. Evans et al. (1951) reported that four of five coagulase positive strains isolated fro· wholesome frozen foods were found to produce enterotoxin. Niven and Evans (1955) stated that all food poisoning staphylococci were coagulase positive. This identification of all enterotoxin producing staphylococci as coagulase positive, however, does not agree with the earlier work of Feldman (1946) or with the work of Thatcher and Simon (1956) who also reported that eterotoxin

may be produced by coagulase negative strains of Micrococcus pyogenes (S. aureus) and from other species of Micrococcus.

4. Numbers of coagulase positive staphylococci required to produce food poisoning

It has been shown above that, in general, coagulase positive staphylococci were responsible for enterotoxin production. The ensuing reports indicate the number of these organisms needed to be present in a food to produce enough enterotoxin to be pathogenic. Evans et al. (1951) stated that foods containing small numbers of coagulase positive staphylococci would appear to be wholesome, but, if such foods were inadequately refrigerated, these organisms could possibly multiply and produce enterotoxin. Hobbs (1953) reported that small numbers of coagulase positive staphylococci and not of a recognized food poisoning type detected only by enrichment cultures could be ignored. She also stated that, in general, those foods suspected of causing food poisoning had counts of greater than 1 million organisms per gram and usually greater than 10 million per gram while the counts for the majority of normal foodstuffs were approximately 10,000 per gram or less. Dack et al. (1960) concluded from a study of naturally occurring staphylococcus food poisoning outbreaks that at least 5 million microorganisms per gram would be required to produce sufficient enterotoxin to cause illness.

5. Competition of staphylococci and salmonellas with other microorganisms

The growth of coagulase positive staphylococci in competition with other microorganisms has been the subject of several reports. In a study of creamed chicken artificially and massively inoculated with S. aureus by Straka and Combes (1952) the food poisoning organisms were outgrown by the saprophytic organisms present. Thatcher and Loit (1961) found that staphylococci were present in low numbers on the skin of chickens and increased very little during storage. Oberhofer and Frazier (1961) noted that the most consistently inhibitory cultures for two enterotoxigenic and two nonenterotoxigenic strains of S. aureus were strains of streptococci (i.e., Streptococcus faecium, S. faecalis, S. faecalis var. liquefaciens, and a nisin producing S. lactis), various meat lactobacilli, and Escherichia coli H 52. Peterson et al. (1962a,b) reported that the greater the saprophytic population, the greater the protection against growth of staphylococci through antagonism, competition for nutrients, and modification of the environment to conditions less favorable for staphylococcal growth. Troller and Frazier (1963) found that S. aureus was inhibited by the following bacteria present in foods: Bacillus cereus, Proteus vulgaris, Serratia marcescens, E. coli H 52, Aerobacter aerogenes, a Pseudomonas spp. isolated from meat, and Achromobacter spp. isolated from

milk. The greatest inhibition occurred at 20° C. (68° F.) to 25° C. (77° F.) and in the range of pH of 6.2 to 7.4 regardless of the inhibitory organisms used.

That salmonellas, like staphylococci, do not usually grow as well as other microorganisms in or on foods is noted from the observation that pre-enrichment and selective inhibition techniques are required for optimum detection of salmonellas from poultry. Inhibition of Salmonella in egg white by E. coli has been demonstrated (Flippin and Mickelson, 1960; Mickelson and Flippin, 1960). Reviews of the methods for the isolation of salmonellas from poultry products have been written by Edwards and Ewing (1962) and by Galton et al. (1964). These specific isolation techniques are designed to allow the salmonellas to multiply at a faster rate than the other bacteria in the food so that when selective media are subsequently used for the detection of the salmonellas, these bacteria will be detected more often than if no enrichment were used.

6. Survival of Staphylococcus aureus and Salmonella species in frozen poultry

None of the common food poisoning microorganisms has been observed to grow below 0° C. (32° F.). In fact, it has been shown that the numbers of these organisms decreased during frozen storage although some were still viable after prolonged

storage periods. Cherry et al. (1946) isolated S. reading from the skin of a turkey that was frozen for more than five months. The lyophilization of S. cholerae-suis did not appear to affect its pathogenicity (Schoening et al., 1949). About 4.4 percent of 911 chickens which had been frozen and stored for an unspecified length of time showed the presence of salmonellas, but the skin of the birds in frozen storage showed a significantly smaller number of salmonellas than that of fresh chilled birds (Schneider and Gunderson, 1949). Thatcher (1958) reported that when turkeys were stuffed with a commercial brand of dehydrated "ready mixed" poultry stuffing that had been inoculated with enterotoxigenic staphylococci or with salmonellas and then frozen at -34.4° C. (-30° F.), enterotoxin developed. The salmonellas multiplied to large numbers, although not until the birds had been out of cold storage for 48 hours. In a study of the effect of freezing methods on the survival of microorganisms on turkeys, Kraft et al. (1963) noted that staphylococci were considerably reduced in number as a result of freezing and holding at -29° C. (-20° F.). These workers also observed that Salmonella survived freezing and frozen storage, but less than one organism per $cm.^2$ was recovered after frozen storage for one month.

The relative importance of salmonellas and staphylococci in foods is summarized by the review of Elliott and Michener

(1961). These authors stated that some pathogens, such as salmonellas or staphylococci, have been shown to be so ubiquitous that their presence in some commercial foods was unavoidable. Caution must therefore be used in the preparation of these foods for consumption, so that they will not become a source of food poisoning or food infection.

D. Effect of Storage Temperatures above 0° C. on Bacteria Recovered from Poultry and Poultry Products

The following reports are concerned with the effect of storing poultry at temperatures above 0° C. (32° F.) on the increases in bacterial counts. Pennington (1911) noted that the bacterial count per gram of flesh of the body wall of chickens continually increased during handling, shipping, and display of the birds at the retail level, even though the temperature was kept below 7.2° C. (45° F.). She also reported that counts for fully eviscerated birds were initially greater and increased more rapidly than those of uneviscerated chickens. Lochhead and Landerkin (1935) found that the deterioration of dressed poultry held at 0° C. (32° F.) was essentially a surface spoilage and that a count exceeding approximately 2.5 million per cm.^{2} was associated with the first signs of surface odors. The results reported by Koonz (1945) were similar to those of Pennington in that the eviscerated stock usually showed about the same bacterial count on the

third day of storage at 10° C. (50° F.) as the New York dressed poultry showed on the fourth day. With regard to organoleptic quality, however, the New York dressed poultry deteriorated more rapidly than the eviscerated poultry, primarily because of the development of visceral taints.

Ayres et al. (1950) concluded that off odor in chicken meat occurred within three days at 10° C. (50° F.), seven days at 4.4° C. (40° F.) and not until 16 days when the poultry was held at 0° C. (32° F.). Baker et al. (1956) reported that bacterial counts on ready-to-cook poultry were highest for birds stored at 7.2° C. (45° F.), intermediate for those stored at 1.7° C. (35° F.) and lowest for those stored at 0° C. (32° F.) in ice. Off flavor first appeared in the liver, gizzard, thigh, and oyster muscle (gluteus medius). In fryers stored in crushed ice at 2° C. (35.6° F.) for 20 days, Mallmann et al. (1958) showed that the bacterial population constantly increased during storage for 17 days.

Studies have also been made on the bacterial counts of the water used for chilling poultry. In experiments performed by Cook (1939), it was noted that the bacterial content of the water in the chill tank at 0° C. (32° F.) increased about 10 times after immersion of five successive lots of chickens, while the bacterial counts of water at 7.2° C. (45° F.) increased about 100 times. Roberts and Robertson (1941) found that bacterial growth in chill water held at 0.6° C. (33° F.)

was extremely slow during the one and one half month testing period. Koonz (1945) also reported that bacterial growth was delayed in water held at 0.6° C. (33° F.) and that the same water could be used for several successive lots of poultry provided that the water was kept at about 1.7° C. (35° F.) or lower.

It is well established that eviscerated chickens stored under refrigeration are usually spoiled by Pseudomonas and Achromobacter. Lochhead and Landerkin (1935) learned that the predominant types of bacteria developing on the skin of chickens during storage were representative of the genera Micrococcus, Flavobacterium, and Achromobacter. It was noted by Ayres et al. (1950) that, at the time chickens were considered to be spoiled, the bacterial flora consisted predominantly of Pseudomonas, Gram negative cocci, and Gram negative coccobacilli. Ayres et al. (1956) determined that after chicken was stored for 12 days at 4.5° C. (40° F.) the psychrophilic population on the chicken meat was 90 percent "Pseudomonas - Achromobacter". Walker and Ayres (1956) indicated that spoilage of poultry stored at 4.4° C. (40° F.) generally occurred within 4 to 6 days and was associated with organisms with characteristics of the "Pseudomonas - Achromobacter group". In a study of fryer chickens stored at 2° C. for 20 days, Mallmann et al. (1958) found that the total count prior to refrigeration was about 15 times higher than the

psychrophilic count, but during storage the difference became less until at the time of spoilage, the counts were identical. Most of the psychrophiles present were Gram negative bacteria. Barnes and Shrimpton (1958) listed the following psychrophilic bacteria on chilled eviscerated chickens stored at 1° C. (34° F.) until spoiled: about 84 percent Pseudomonas spp., about 13 percent Achromobacter spp., and about 3 percent miscellaneous types including Cytophaga spp. and Flavobacterium spp.

Of 103 cultures isolated from chilled, cut-up fryers which had been allowed to spoil at 4.4° C. (40° F.), Nagel et al. (1960) found that 88 were Pseudomonas spp., 13 were placed in the "Achromobacter - Alcaligenes group" and two were Aeromonas spp. These investigators also determined that the geographical location of the processing facilities and the location of the cutting-up operations had no influence on the distribution of the genera of spoilage organisms. Barnes (1960a,b) observed that Pseudomonas were the main spoilage organisms of chilled, eviscerated poultry. Thornley (1960) noted that, of 391 Gram negative bacteria isolated from chicken meat spoiled at 1° C. (34° F.), 156 were Pseudomonas spp., 188 were Achromobacter spp., and 47 others belonged to the coli-aerogenes group or remained unclassified. Thornley et al. (1960) indicated that the "Pseudomonas - Achromobacter group" initially formed about 16 percent of the population on fresh eviscerated chickens. When chickens were stored at 1° C.

(34° F.) until spoilage occurred, these organisms grew and eventually predominated on the spoiled carcasses.

If eviscerated poultry is kept at 5.5° C. (42° F.) or below, there probably would be little possibility of multiplication of salmonellas or staphylococci. Working with chicken a la king, Angelotti et al. (1961) concluded that neither type or organisms displayed any important change in numbers of cells per gram throughout a 5-day incubation period at 4.4° C. (40° F.) or 5.5° C. (42° F.). However, at 6.5° C. (44° F.) increases were noted in both groups after incubation for 4 days.

E. Effect of Storage Temperatures below 0° C. on Bacteria Recovered from Poultry and Poultry Products

The effect of freezing and frozen storage on microorganisms has been reviewed by several workers (Sedgwick and Winslow, 1902; Belehradek, 1935; Luyet and Gehenio, 1940; Ingram, 1951; Borgstrom, 1955). Ingram summarized the known effects as follows:

(a) There was a sudden mortality immediately on freezing, varying with species.
(b) The proportion of cells surviving immediately after freezing was nearly independent of the rate of freezing.
(c) The cells which were still viable immediately after freezing died gradually on storage in the frozen state.

He also pointed out that freezing and frozen storage prevented microbial activity.

Several studies of poultry and poultry products have confirmed the above findings. Gunderson et al. (1947) isolated only nine genera of microorganisms from eviscerated poultry which had been stored in the frozen state in contrast to 21 genera that had been isolated from the surface of freshly killed chickens. During storage at -25.5° C. (-14° F.) for periods ranging from 30 to 280 days, the total bacterial count in boned chicken and chicken chow mein showed a rather sharp reduction in the coliform flora, but a slow decrease in the total flora (Gunderson and Rose, 1948).

Sair and Cook (1938) concluded that from the standpoint of the bacterial numbers in properly handled poultry quick freezing had no advantage over slow freezing. A later report (Kraft et al., 1963) showed that freezing poultry by air blast was more effective in reducing total bacterial numbers on the skin surfaces of uninoculated turkeys than was brine immersion followed by air blast. These workers also found that the numbers of viable organisms decreased at a much slower rate when turkeys were held in frozen storage than during the original freezing operation.

Reports (Sulzbacher, 1950; Hartsell, 1951; Wilkerson et al., 1961) have shown that total aerobic bacterial counts sometimes increased during frozen storage. Hartsell accounted for this phenomenon by stating that apparently subfreezing temperature altered the physiological states of the surviving

cells so that a much shorter generation time occurred. The reason for this stimulus was not apparent. Sulzbacher stated that the increased count could also represent a combination of experimental variables and growth that took place during the cooling period after the samples were placed in the freezer.

III. MATERIALS AND METHODS

A. Collection of Samples in the Processing Plants

1. Description of processing plants visited

Samples were collected from five federally inspected turkey processing plants. Four of these plants were located in eastern or central Iowa and one was in southern Minnesota. Four of the factories produced further processed turkey products as well as whole frozen turkeys. The other plant produced only whole frozen turkeys.

Plant A in Minnesota produced about 50 percent whole frozen turkeys and 50 percent turkey products including rolls, roasts, parts and stuffed turkeys. The average number of birds slaughtered per day was 7500.

The major products in Plant B were turkey rolls and roasts. On an average day 2500 turkeys were processed; the maximum load exceeded 4000 birds per day.

Processing Plant C prepared whole frozen turkeys exclusively. The size of the operation in this plant was 4500 turkeys on a normal day and 6000 birds during the height of the processing season.

The primary product in Plant D was frozen whole birds; some roasts and rolls were also produced. This plant usually processed 10,000 birds, with a maximum kill of 13,500 turkeys

daily.

Plant E produced mainly frozen turkeys and to a lesser extent turkey parts, rolls, and roasts. The number of turkeys prepared in the plant was 10,000 a day during the peak season and 8,000 birds on an average day.

In Plant A, the livers and hearts were washed in one tumbler washer while the gizzards were washed in a second tumbler washer. In Plant B, the livers were collected in a perforated pan. Water flowed continuously over most of the livers contained in this pan. At intervals, the livers were removed to be iced and empty trays were placed under the faucet. Hearts were treated in the same manner as the livers, but gizzards were sent through a tumbler washer having an overhead water spray. In Plant C, all of the giblets were conveyed by flume from the evisceration station to the wrapping table. Water was introduced and then drained at three points on the flume. In Plant D, all the giblets were flumed from the evisceration line to the packaging station with one change in water. In Plant E, livers and hearts were washed in tumbler washers, while the gizzards were washed by hand. In Plants A, C, and D, the giblets were packaged after washing and then iced. In Plant B, the livers, hearts and gizzards were iced in separate pails and then frozen in the pails. In Plant E, the giblets were iced overnight and then packaged.

2. Sampling procedures

a. Uninoculated samples During the 1962 processing season, samples were collected only from Plant E. Swab samples from the processing line were obtained during the first and second visit by use of a technique in which a moistened swab was rolled over a 2 $cm.^2$ area. The cotton portions of the swab sticks were then snapped off into tubes containing 10 ml. of 0.1 percent Bacto* peptone in distilled water. Upon returning to the laboratory the iced tubes were sampled for populations of total aerobes, coliforms, enterococci, staphylococci, and salmonellas.

On the first visit to Plant C, samples were taken from the following:

a) giblets (gizzards, hearts, and livers) swabbed after the visceral cavity had been opened and the viscera pulled out, but before federal inspection;

b) giblets sampled after being cut from the birds;

c) giblets sampled after inspection and washing;

d) gizzards sampled after being stripped by machine.

The plant had not been operating continuously at the time of the first visit and no samples were obtained after the giblets had been iced for 24 hours.

On the second trip, samples were collected from the

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*Difco Laboratories, Detroit, Michigan.

following:

a) giblets (gizzards, hearts, and livers) swabbed after the visceral cavity had been opened and the viscera pulled out, but before federal inspection;

b) giblets swabbed after federal inspection and before being cut from the birds;

c) giblets sampled after federal inspection and washing;

d) giblets swabbed after being held in ice for 24 hours and immediately before being placed in pouches.

On the third, fourth, and fifth visits, samples a), c), and d) were the same as those used on the second visit. The b) samples were obtained from the livers and hearts immediately after these organs were cut from the birds and from the gizzards after stripping. On each visit, three samples were taken from each type of organ at each station.

On five subsequent trips to Plant E during 1962, swab samples were taken from gizzards and livers before washing and after washing. Ten organs of each type were examined after the livers were removed from the birds, after the gizzards were stripped, and after the livers and gizzards had been washed. Two $cm.^2$ areas of the convex (parietal) surface of the livers and fleshy portions of the gizzards were swabbed for total aerobic counts. After the two $cm.^2$ areas had been sampled, the entire convex surface of livers and the glandular mucosa of gizzards were swabbed for differential counts. All

swabs used for sampling were placed into 10 ml. of a 0.1 percent solution of peptone.

During the 1963 processing season, five visits each were made to Plants B, C, and D. The methods employed were identical with those described previously except that 20 giblets of each type were used instead of 10. The procedure on the last visit to these plants was also varied in that swabbings were made from the entire convex surface of livers other than those swabbed for total and differential counts. These swabs from this second group of livers were transferred to 10 ml. of lactose broth. The lactose broth was a pre-enrichment medium used for Salmonellae detection. Two visits were also made to Plant A. The procedures used on these trips were identical with those used on the last trip to Plants B, C, and D, except that 30 unwashed and 30 washed livers were collected on each visit instead of 20 of each kind.

During the 1964 processing season, two visits were made to Plants B, C, and D, and three visits were made to Plant E. On each trip, the entire convex surface of 20 unwashed and 20 washed livers was sampled by swab technique. These swabs were transferred into 10 ml. of lactose broth.

b. Inoculated samples Studies with livers inoculated with Serratia marcescens (2G12)* were made in Plant E. Livers

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*Department of Bacteriology, Iowa State University, Ames, Iowa.

were dipped into a suspension of the inoculum, allowed to drain, and washed in the tumbler washer in the plant. Immediately after these livers were washed, uninoculated livers were washed and collected. Livers were also gathered every hour for 5 to 6 hours after the inoculated samples had been washed. These livers were placed in polyethylene bags and held in ice until they could be examined in the laboratory the next day. Size of inoculum was determined by sampling other livers which were dipped into the suspension of organisms and then drained.

B. Laboratory Sampling of Giblets

1. Sampling frozen products

a. Uninoculated samples Unwashed hearts, livers, and stripped gizzards were taken from the processing line of Plant E at approximately two week intervals beginning in June, 1962. A total of seven collections were made. At each visit, six giblets of a given type were placed in a polyethylene pouch by the factory worker. A total of 30 hearts, 30 livers, and 30 gizzards were taken on each visit.

The giblet samples were brought to the laboratory and frozen at -29° C. in a walk-in blast type freezer located in the Food Processing Laboratory. These samples were maintained at -29° C. until examined. The giblets were removed from the

freezer and examined at intervals of approximately 1 month, 2 months, 4 months, 8 months, and 12 months after being frozen.

One pouch each of hearts, livers, and gizzards was placed in tepid water (35-40° C.; 95-104° F.) and thawed for 1 to 2 hours. Bacterial examinations were made of the giblets using the swab technique. Determinations were made of the numbers of total aerobes, coliforms, enterococci, and coagulase positive staphylococci. Studies were also performed to detect the presence of salmonellas.

For the examination of giblets which were obtained from the first four collections and stored for one month, and for the tests after two months' storage of giblets from the first collection, a 2 $cm.^2$ area of each giblet was swabbed for all total and differential counts. For the remainder of the counts in this study of frozen giblets, the swabbing procedure was identical to that described for sampling fresh giblets.

b. Inoculated samples In the laboratory, livers were dipped into a peptone water suspension of S. marcescens (2G12), allowed to drain and then washed in a tumbler washer for 5 minutes (Figure 1). The washer was constructed from 1/4 inch mesh screening bent to form a cylinder with a diameter of 6 inches. A rod passing horizontally through the center of the cylinder was attached to a pulley. The pulley was driven by a V belt connected to an electric motor and the speed of

Figure 1. Laboratory giblet washer

the washer was set at 35-40 r.p.m. The washer was placed in a tub and the tub was filled with cold water (about 5° C.; 41° F.) to a depth 1 1/2 inches above the bottom of the washer. The water was not changed during the washing of uninoculated livers for 5 minutes. The size of the inoculum was determined in the same manner as described for plant washing of inoculated livers.

C. Determination of the Area of Giblet Surfaces

In order to determine the approximate area of the surfaces of giblets that were swabbed, a piece of Saran Wrap* was placed over the surface and the excess film cut away from the edge of the giblet. The outline of the film remaining was traced onto graph paper and the area of the tracing was determined (Figure 2).

D. Media Employed

Plates for total counts were poured with trypticase soy agar (B.B.L.).** These plates were incubated for 5 days at 15° C. This medium was also used for the counts of the indicator organism, S. marcescens (2G12). After incubation at

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*Dow Chemical Company, Midland, Michigan.

**Baltimore Biological Laboratories, Baltimore, Maryland.

Figure 2. Method for determining the surface area of giblets


SARAN WRAP
SARAN WRAP

30° C. (86° F.) for 48 hours, all colonies which had a red pigment were counted.

Numbers of enterococci were estimated by plate count, using KF streptococcus agar (Difco). Coliforms were enumerated on violet red bile agar (VRB, Difco). Media for counts of coliforms and enterococci were incubated at 37° C. (98° F.) for 24 to 48 hours.

The presence of possible coagulase positive staphylococci was determined by spreading 0.1 ml. or 0.25 ml. aliquots of the peptone water on plates of staphylococcus medium no. 110 (Difco) fortified with egg yolk (Herman and Morelli, 1960). After incubation at 30° C. (86° F.) for 72 hours, colonies surrounded by zones of precipitation and clearing of the yolk were inoculated into brain heart infusion broth (Difco) and incubated for 18-24 hours at 37° C. (98° F.). A tube coagulase test was performed on these cultures using Bacto coagulase plasma (Difco). The results were read after incubation for 1 and for 3 hours in a water bath at 37° C.

The method of detecting salmonellas suggested by the Institute of American Poultry Industries (1960) was used except that bismuth sulfite agar was not included. In later tests, triple sugar iron agar (Difco) was substituted for dulcitol lysine iron agar, because hydrogen sulfide production was more pronounced in the former medium. Confirmation of positive samples was made on lysine-iron agar (Edwards and

Fife, 1961). The cultures were then sent to the National Animal Disease Laboratory, Ames, Iowa, or the Communicable Disease Center, Atlanta, Georgia, for identification of species.

Cultures isolated from processed giblets were streaked on nutrient agar (BBL) containing 10 percent sterile skim milk (Matrix Mother Culture Media).* These plates were incubated at 30° C. (86° F.) for 48 hours. Stock cultures were streaked on stock culture agar (Difco), incubated at 30° C. for one or two days and then held under refrigeration. Nutrient agar was used as a general plating medium. Other media used in the identification of the unknown cultures included OF basal medium (Difco), OF basal medium plus 1 percent sterile glucose, nitrate broth, MR-VP medium (Difco), peptone water (Edwards and Ewing, 1962), Simmons' citrate agar (Difco), urease test medium (Stuart et al., 1945), eosin methylene blue agar (Difco), KF Streptococcus agar (Difco), Medium B of King et al. (1954), malt agar (Difco), V-8 juice (Campbell Soup Co., Inc.)** medium, tomato juice agar (Difco), and modified Gorodkowa agar (Lodder and Kreger-van Rij, 1952).

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*Galloway-West Company, Fond du Lac, Wisconsin.

**Campbell Soup Co., Camden, New Jersey.

E. Characterization of Microorganisms Isolated from Processed Giblets

Colonies were selected for further study in the following manner. A line was drawn (with a wax marking pencil) down the middle of the bottom of a petri dish containing a representative bacterial count. The ten colonies situated on or closest to this line were picked and streaked onto nutrient agar with skim milk. After incubation, colonies were again transferred by further streaking until pure cultures were obtained. Colonies were picked from plates prepared from the following samples:

1) Two swabbings from unwashed and washed livers from the last visit to Plant B in 1963 (40 cultures), and from the last two visits to Plants C and D in 1963 (160 cultures).
2) Three swab samples from unwashed and washed livers from the visits in 1963 to Plant A (120 cultures).
3) Water from a bucket of livers being chilled in Plant B (eight cultures).

A total of 328 cultures were picked. Of these, 306 were viable after the initial transfer and the following procedures were used to characterize these cultures. The colony appearance on the skim milk agar was noted. Gram stains were made of all cultures and the Gram reaction, size and shape of the organisms were recorded. All cultures were examined for their

ability to grow at 2-3° C. (35.6-37.4° F.) and at 45° C. (112° F.). Some of these bacteria were also tested for growth at 37° C. (98° F.) when considered necessary to aid in classification.

All Gram positive cultures were characterized according to Bergey's Manual of Determinative Bacteriology (Breed et al., 1957). The catalase reaction of these bacteria was determined using 3 percent hydrogen peroxide (Society of American Bacteriologists, 1949). Cultures were characterized further by their ability to oxidize or ferment glucose. The ability of Gram positive rods to reduce nitrate was recorded.

Gram negative bacteria were identified by the schema of Hugh* (1964) (Table 3). These Gram negative bacteria were stab-inoculated into unsealed and sealed (1.5 ml. seal of equal parts by weight of paraffin and mineral oil) tubes of OF basal medium with and without 1 percent glucose. The tubes were incubated at 30° C. for 14 days or less. All cultures which produced acid or alkali in the open tube and no change in the sealed tube of OF basal medium with glucose were inoculated into petri dishes containing OF basal medium. Transfers were made from these plates for flagella staining

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*Hugh, R. H., Washington, D. C. Classification of certain Gram negative non-spore forming rods. Private communication. 1964.

Table 3. Classification of Gram negative non-spore forming rods

Genus	Motility	Flagella	OF glucose	Pigment	Oxidase
Pseudomonas	+	Single; polar or tuft	Oxidative, alkaline, or negative	H_2O soluble (fluorescent) + - or $CHCl_3$ soluble green-yellow or blue	+ -
Aeromonas	+	Single; polar	Fermentative	-	+
Achromobacter	+	Peritrichous	Oxidative	-	
Alcaligenes	+ -	Peritrichous	Alkaline	-	
Flavobacterium	+	Peritrichous	Oxidative	H_2O insoluble orange-yellow	+
Xanthomonas	+	Single; polar	Oxidative	H_2O insoluble yellow	
Comamonas	+ -	Lophotrichous	Alkaline	-	
Enterobacteriaceae	+ -	Peritrichous	Fermentative		

by the method of Rhodes (1958). Cultures exhibiting polar flagella were inoculated on Medium B of King et al. (1954). An oxidase test (Kovacs, 1956) was made on cultures which produced acid in both the open and sealed tubes of OF basal medium containing added glucose. If the oxidase test was negative, these cultures were identified in accordance with the schema proposed by Edwards and Ewing (1962). These isolates were tested for the production of indole, acid and acetylmethylcarbinol from MR-VP medium, growth on Simmons' citrate agar, hydrogen sulfide, ammonia from urea, and acid from arabinose.

Identification of the yeasts isolated followed the plan of Lodder and Kreger-van Rij (1952).

IV. RESULTS

A. Effect of Processing on the Bacteria of Commercially Prepared Giblets

1. Comparison of giblets and of sampling stations

In order to determine if there were differences in numbers of bacteria on gizzards, hearts, and livers during various stages of processing, in Plant E, swab samples were collected at the four processing stations previously described. The results of the total aerobic bacterial counts appear in Table 4. It can be determined from this table that although the counts were variable, the greatest change in number of organisms usually occurred during the washing process. It can also be seen that, in general, the counts obtained from the surface of turkey hearts were similar to those from the livers. Coliform counts were in a range of 0 to 228 organisms per $cm.^2$ with most plates showing no coliforms (Table 5). Enterococci varied from 0 to 250 organisms per $cm.^2$ with the majority of the counts less than 50 per $cm.^2$ (Table 6). Enterococci were detected on about 30 percent of the samples for which the coliform counts were negative and in other cases were usually present in higher numbers than coliforms. A trend of a decrease in count due to washing could be determined from the numbers of these fecal indicator organisms.

Table 4. Median of total aerobic bacteria per cm.^{2} recovered from turkey giblet surfaces at four processing stations

Visit	After visceral cavity opened and viscera pulled out	After removal, but before washing	After washing	After 24 hours
		Gizzards		
A	350	310	130	
B	1,090	2,300	70	130
C	4,250	890	890	4,350
D	3,150	280	280	410
E	3,750	2,450	80	7,890
		Hearts		
A	280	260	150	
B	240	610	50	90
C	1,140	210	80	800
D	650	640	60	80
E	8,430	860	150	580
		Livers		
A	490	490	100	
B	2,350	430	170	120
C	640	970	80	240
D	620	1,480	50	280
E	800	1,050	300	380

Table 5. Median of coliform bacteria per $cm.^2$ recovered from turkey giblet surfaces at four processing stations

Visit	After visceral cavity opened and viscera pulled out	After removal, but before washing	After washing	After 24 hours
		Gizzards		
A	30	20	<1	
B	80	30	<1	<1
C	50	5	10	5
D	30	<1	8	3
E	30	3	<1	<1
		Hearts		
A	10	10	<1	
B	10	20	<1	<1
C	8	<1	<1	<1
D	<1	<1	<1	<1
E	10	3	3	<1
		Livers		
A	40	10	<1	
B	60	40	<1	<1
C	8	5	<1	<1
D	<1	230	<1	<1
E	3	3	<1	<1

Table 6. Median of the enterococcal count per cm.^{2} recovered from turkey giblet surfaces at four processing stations

Visit	After visceral cavity opened and viscera pulled out	After removal, but before washing	After washing	After 24 hours
		Gizzards		
A	20	60	<1	
B	50	40	8	<1
C	20	8	20	120
D	230	30	5	70
E	30	30	5	20
		Hearts		
A	40	230	<1	
B	<1	40	<1	<1
C	30	<1	<1	3
D	90	270	<1	3
E	420	3	<1	20
		Livers		
A	70	250	<1	
B	<1	<1	<1	<1
C	20	20	<1	3
D	20	450	<1	3
E	30	10	<1	<1

It was decided to concentrate the survey on the effect of washing on bacterial counts on the surface of livers and gizzards.

2. Role of washing in reducing bacterial numbers

a. Uninoculated giblets During the 1962 processing season, giblets at processing Plant E were examined for total aerobic bacteria, enterococci and coliforms. The results of these experiments are shown in Table 7. In order to convert all counts to a $cm.^2$ basis, the area of the convex (parietal) surface of livers and the area of the glandular mucosa of gizzards were measured as described in the section on methods. The liver surface was about 80 $cm.^2$ and the surface of the gizzard samples was approximately 55 $cm.^2$.

Bacterial populations ranged from 5 to 7400 per $cm.^2$ on the surface of livers and from 10 to 8000 per $cm.^2$ on the surface of gizzards (Table 7). The population of coliforms on the livers varied from 0 to 38 per $cm.^2$; the number of enterococci ranged from 0 to 100 per $cm.^2$. On gizzards, enterococci were found in a range from 0 to 36 per $cm.^2$ and the coliforms were recovered in numbers from 0 to 22 per $cm.^2$. The data presented in Table 7 show that the total aerobic population had little or no change due to processing while the fecal indicator organisms decreased during processing.

Table 7. Median count of the total aerobic bacteria, coliforms and enterococci per cm.^{2} of turkey gizzards and liver surface sampled in Plant E

Visit	Total aerobes		Coliforms		Enterococci	
	Before washing	After washing	Before washing	After washing	Before washing	After washing
			Gizzards			
A	225	678	0.9	0.5	9.8	0.7
B	268	113	3.0	0.1	15.9	0.7
C	825	525	0.6	0.5	6.2	1.3
D	423	80	0.3	<0.1[a]	0.6	<0.1
E	588	218	0.6	0.1	0.8	0.2
			Livers			
A	853	290	9.9	1.0	5.2	0.5
B	250	95	4.1	0.5	36.9	0.1
C	310	583	3.8	0.1	15.9	0.6
D	203	528	0.4	0.1	0.6	0.1
E	685	65	12.6	0.3	24.6	0.5

[a]No colonies detected at lowest dilution.

During the 1963 turkey processing season, samples were obtained from giblets processed in Plants A, B, C, and D. The results of the bacterial counts on the surface of giblets are shown in Figures 3, 4, and 5. The results of the total aerobic counts indicated that, before washing, 71-85 percent of all the counts were in the range of 10-1000 organisms per

Figure 3. Incidence of total aerobic bacteria on turkey giblets from four processing plants

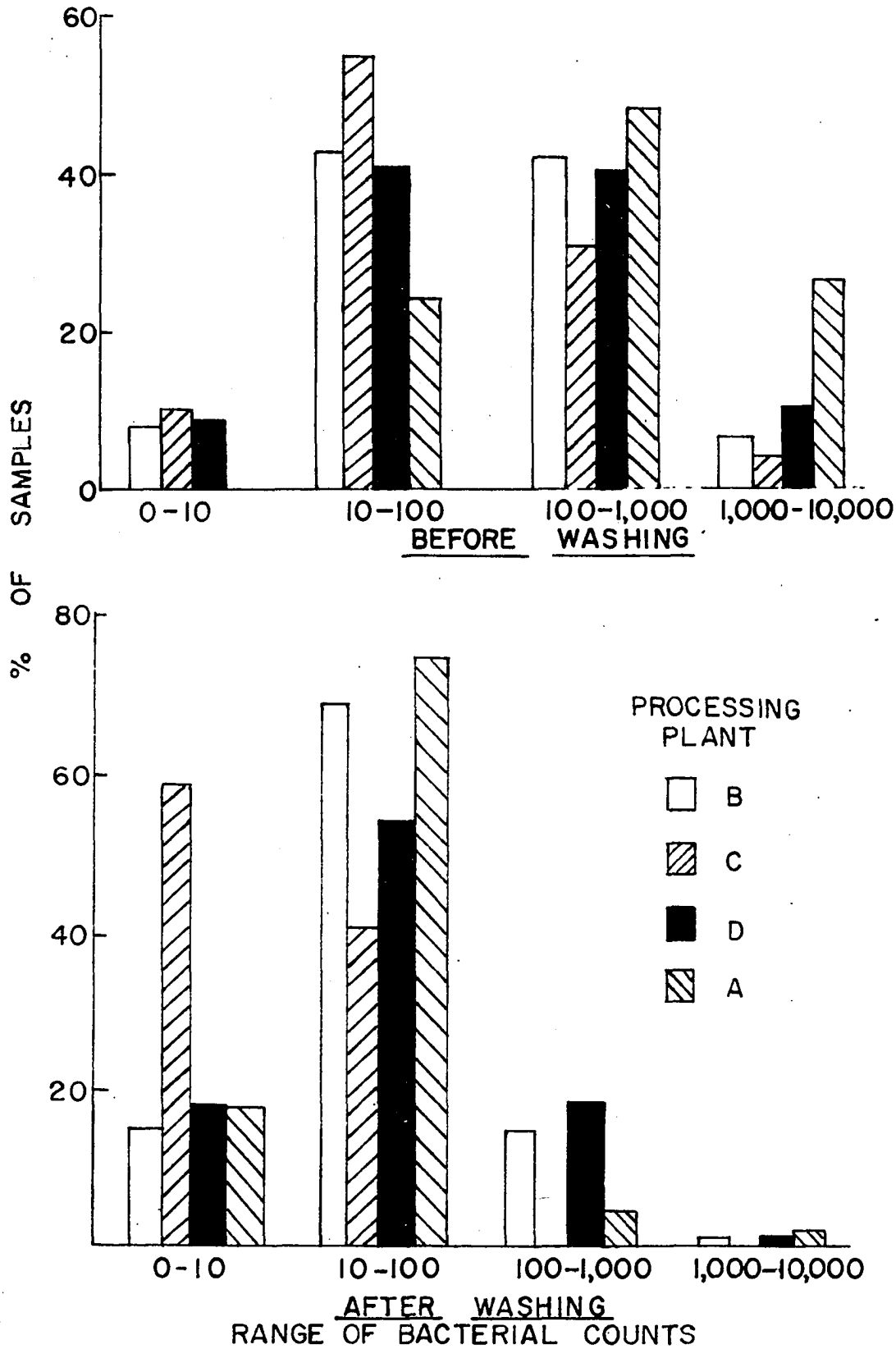

60
40
20
0
% OF SAMPLES
0-10
10-100
100-1,000
1,000-10,000
BEFORE WASHING
80
60
40
20
PROCESSING PLANT
B
C
D
A
0-10
10-100
100-1,000
1,000-10,000
AFTER WASHING
RANGE OF BACTERIAL COUNTS

Figure 4. Incidence of coliform bacteria on turkey giblets from four processing plants

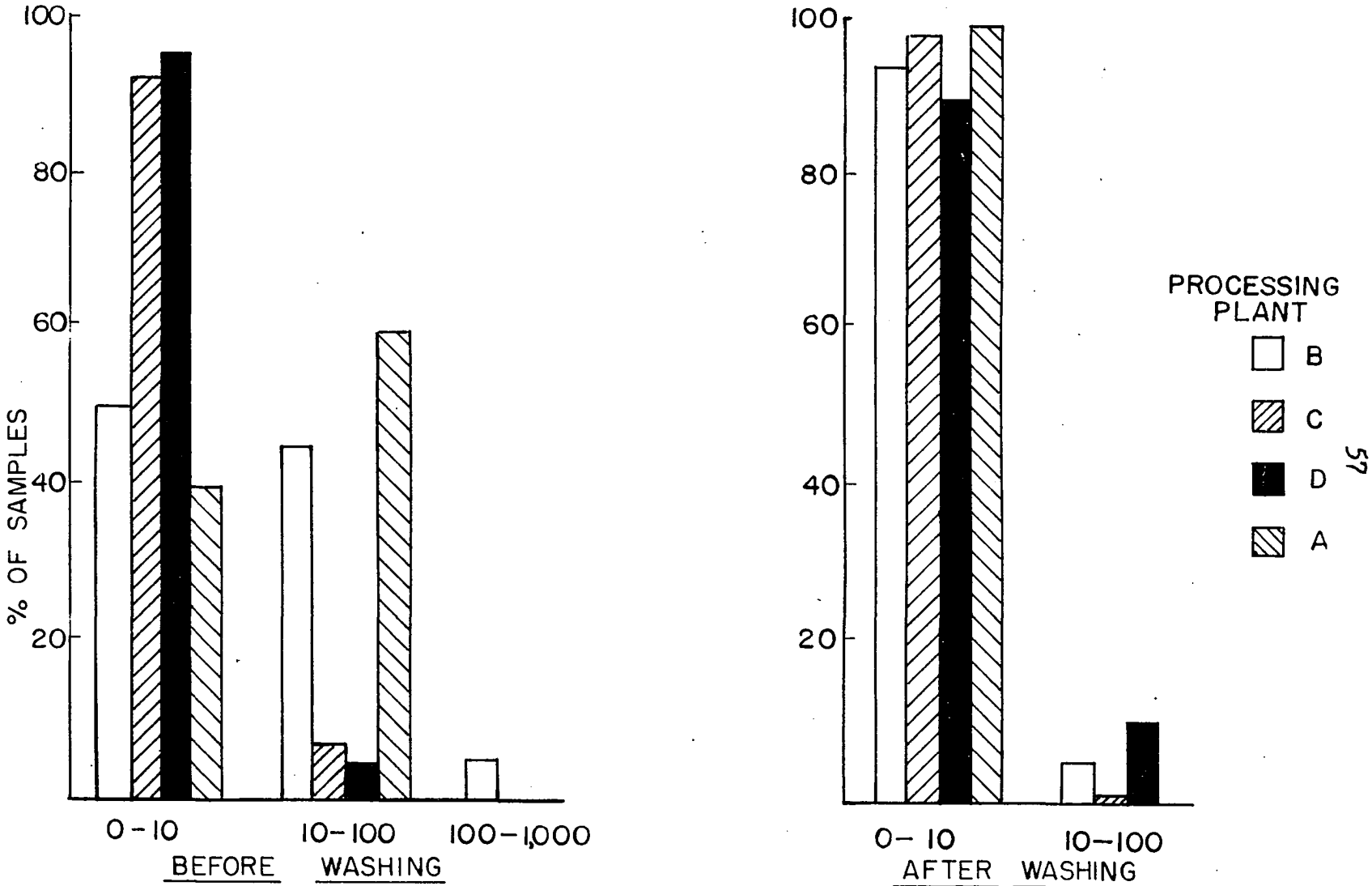
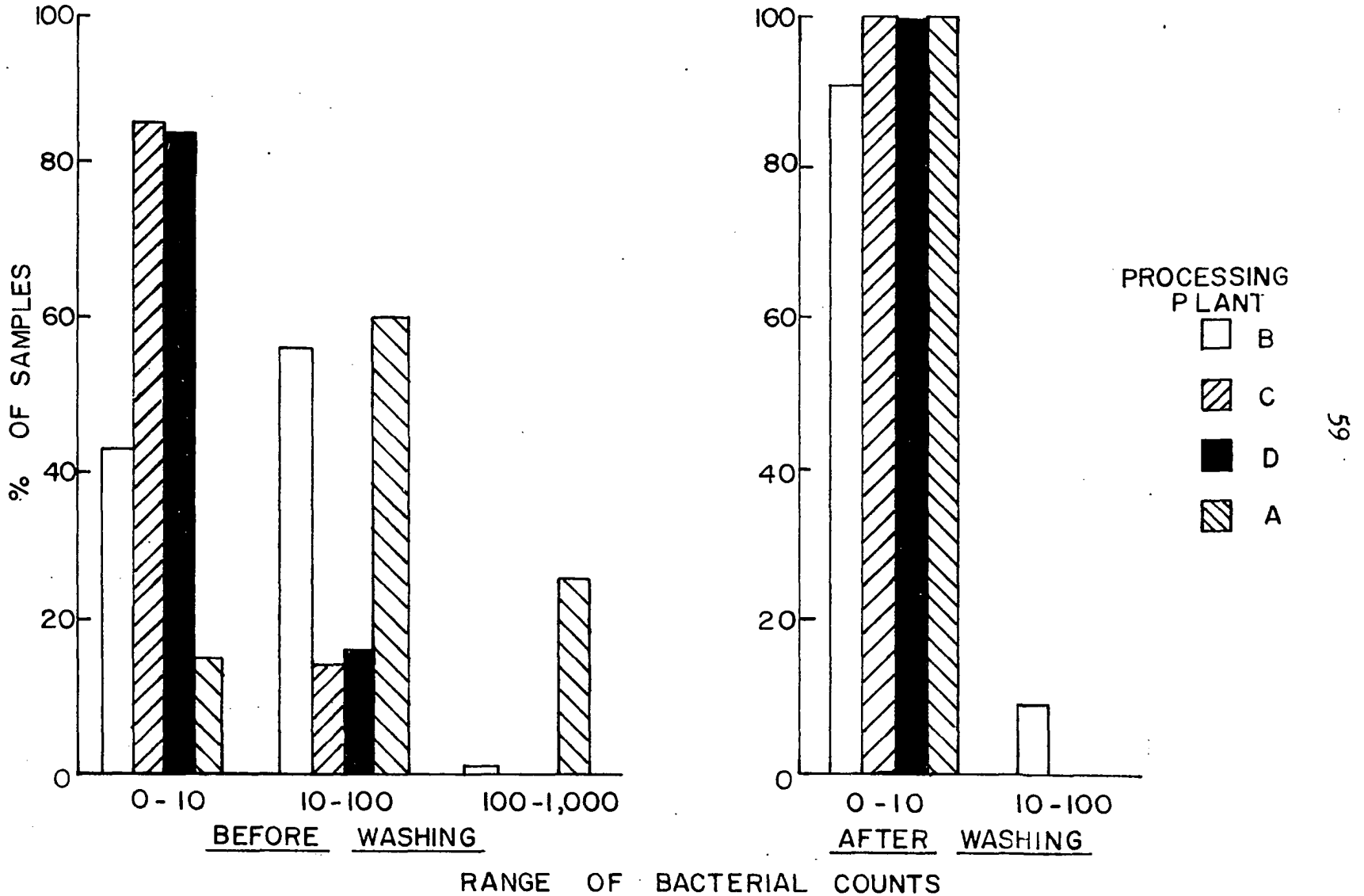

100
80
60
40
20
% OF SAMPLES
0-10
10-100
100-1,000
BEFORE WASHING
100
80
60
40
20
0-10
10-100
AFTER WASHING
PROCESSING PLANT
B
C
D
A
RANGE OF BACTERIAL COUNTS

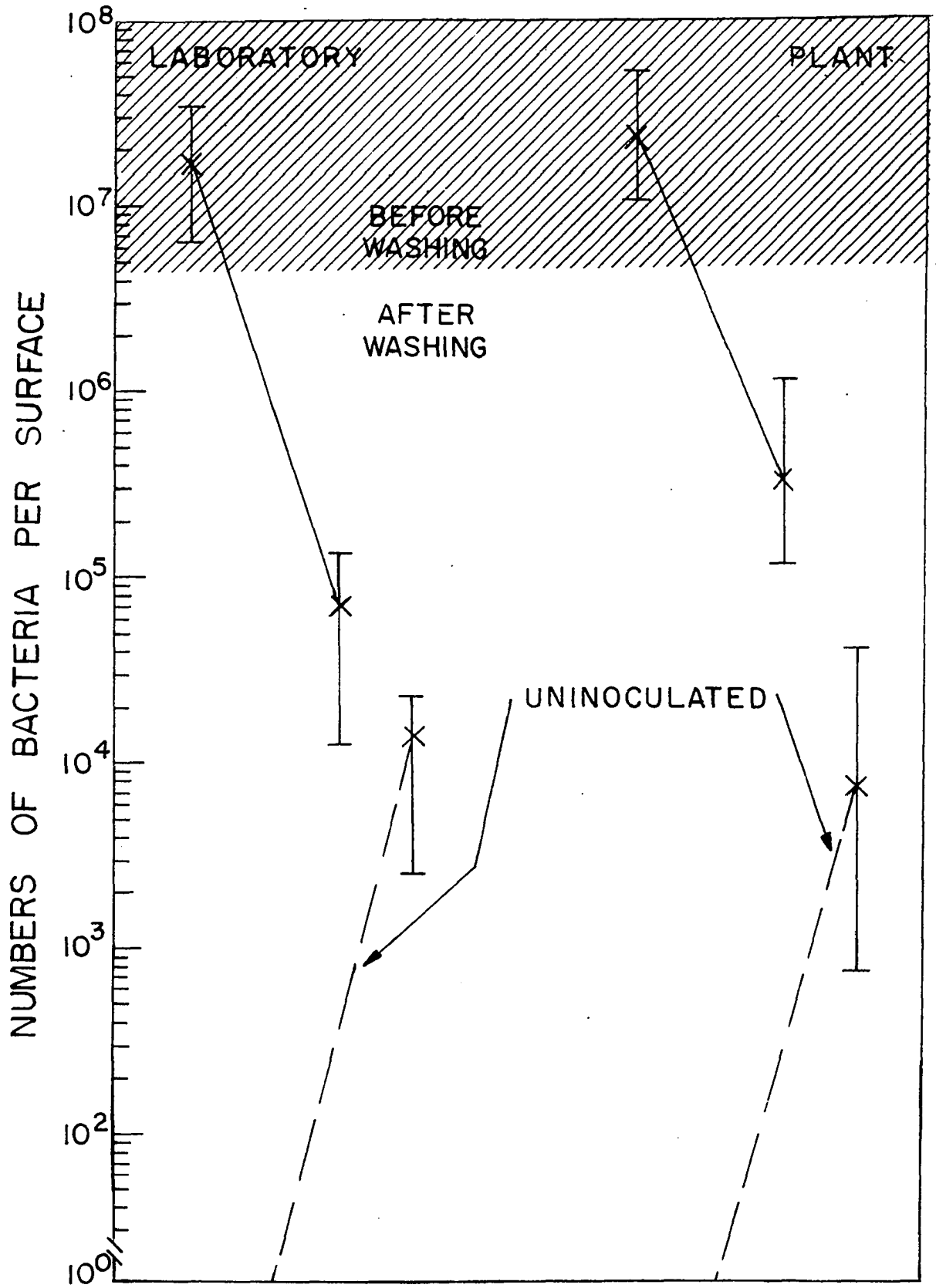
Figure 5. Incidence of enterococci on turkey giblets from four processing plants


100
80
60
40
20
0
% OF SAMPLES
0 - 10
10 - 100
100 - 1,000
BEFORE WASHING
0 - 10
10 - 100
AFTER WASHING
RANGE OF BACTERIAL COUNTS
PROCESSING PLANT
B
C
D
A

cm.^{2}, while after washing 72-100 percent of the counts from all plants were in the range of 0-100 organisms per cm.^{2} (Figure 3). The results of the coliform counts revealed that 95-100 percent of these counts were in the range of 0-100 organisms per cm.^{2} on the unwashed livers and that 90-100 percent of the counts occurred in the range of 0-10 organisms per cm.^{2} on the surface of washed giblets (Figure 4). Figure 5 illustrates that the distribution of enterococci was similar to that of coliforms from all processing plants. It can be seen from these data that washing served to provide at least a 10-fold reduction in numbers of fecal indicator and total aerobic organisms.

b. Inoculated giblets Since washing did not result in a decrease in the number of total aerobes but did reduce the population of fecal indicator organisms in Plant E, studies with livers inoculated with Serratia marcescens were performed to determine if washing actually reduced the bacterial population or if recontamination resulted from the washing procedure. Figure 6 depicts the reduction due to washing in the laboratory and in the processing plant on numbers of bacteria on livers dipped in a high level of inoculum of about 10,000,000 organisms. The figure also shows the subsequent contamination of uninoculated livers. The short vertical lines indicate the zones within which the counts ranged. Numbers of S. marcescens were greatly reduced after

Figure 6. Effect of washing on numbers of bacteria on livers dipped in a high level of inoculum and subsequent contamination of uninoculated livers


LABORATORY
PLANT
BEFORE WASHING
AFTER WASHING
UNINOCULATED
NUMBERS OF BACTERIA PER SURFACE
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0

washing. Uninoculated livers were then contaminated by the inoculum in the water to yield counts in the range of 700 to 40,000 per cm.^{2}. Similar trends were found when a level of inoculum of about 100,000 to 1,000,000 bacteria was used (Figure 7).

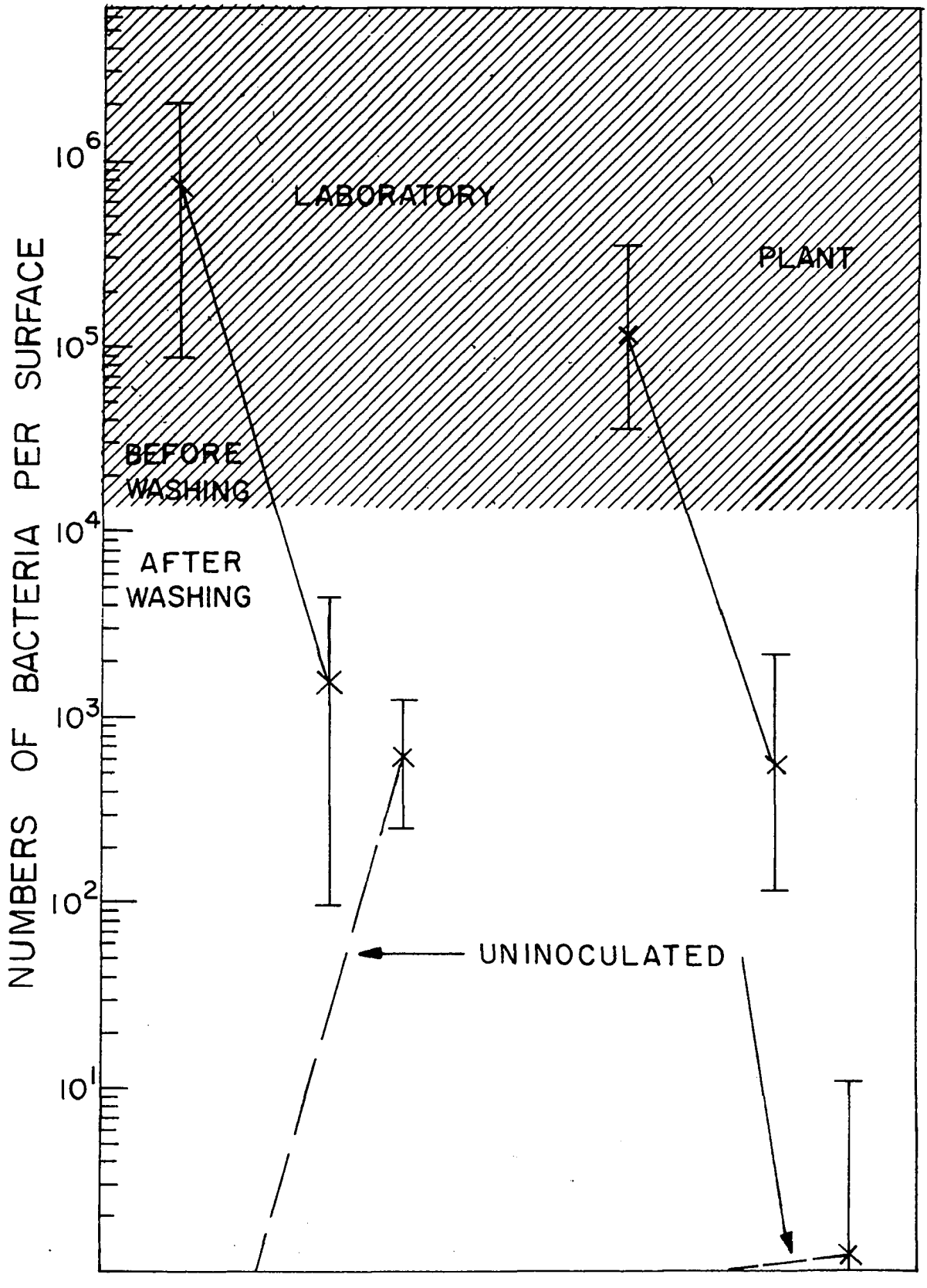
The length of time that the inoculum could be detected on previously uninoculated livers passing through the washer was also determined. Forty-five minutes after livers bearing millions of S. marcescens had been washed, this organism was recovered at a level of 10 to 1100 bacteria per liver. After 2 hours, none of these test organisms were detected, nor were any recovered later during the day.

3. Incidence of coagulase positive Staphylococcus aureus and Salmonella species

In processing Plant E, where both gizzards and livers were sampled, coagulase positive staphylococci were recovered from four of 50 gizzards before washing. In each instance only 10 organisms per sample were recovered. None of these bacteria were isolated from any of the 50 washed gizzards, or from the 50 unwashed and 50 washed livers.

In Plants A, B, C, and D, where unwashed and washed livers were examined, samples positive for coagulase positive staphylococci were detected from the unwashed livers on two visits to every processing plant. In two processing plants

Figure 7. Effect of washing on numbers of bacteria on livers dipped in a low level of inoculum and subsequent contamination of uninoculated livers


NUMBERS OF BACTERIA PER SURFACE
10^6
10^5
10^4
10^3
10^2
10^1
LABORATORY
PLANT
BEFORE WASHING
AFTER WASHING
UNINOCULATED

they were also recovered from the surface of washed livers which were sampled that day. The number of isolations from each processing plant can be seen in Table 8. Numbers of coagulase positive staphylococci isolated from these livers varied from 50 to 700 per liver surface. Increasing the size of the inoculum from 0.1 ml. to 0.25 ml. could have accounted for the increase in the number of recoveries of coagulase positive staphylococci.

Table 8. Isolations of coagulase positive staphylococci from turkey giblets

Processing plant	Visit number	Positive samples		Total samples
		Unwashed livers	Washed livers	
A[a]	1	12	1	60
	2	26	0	60
B	1	2	0	40
	4	4	0	40
C	4	9	2	40
	5	2	0	40
D	4	2	0	40
	5	5	0	40

[a]Size of all samples in this plant was increased from an aliquot of 0.1 ml. to an aliquot of 0.25 ml. of peptone water inoculum.

A Salmonella survey (Table 9) showed that only one of 680 swab samples used for detecting salmonellas was positive when swabs were first broken into peptone water and then the inoculum added to lactose broth. However, 49 of 600 samples were positive for salmonellas when the swab was broken directly into a tube of lactose broth. These results indicated that salmonellas were sometimes present on the surface of giblets and if a sufficiently large sample had not been taken they would be undetected.

B. Effect of Frozen Storage

Tables 10, 11, and 12 present the medians of the counts per cm.^{2} of total viable aerobes, coliforms, and enterococci respectively. The total aerobic counts did not show the general decline with time which would be expected during frozen storage (Table 10). It is also noted that, while the median total population of the fresh unwashed giblets was in a range of 200 to 2,450 organisms per cm.^{2}, the counts of the frozen giblets varied much more widely and for several samples the median exceeded 10,000 bacteria per cm.^{2}. The coliform counts, on the contrary, were not greater during frozen storage than they were on the fresh unwashed giblets (Table 11). In fact, most of the coliform counts obtained from gizzards and hearts were very low during frozen storage, but numbers of coliforms on the frozen livers generally were

Table 9. Number of isolations and species of *Salmonella* recovered from turkey livers processed in five plants

Processing plant	*Salmonella* species	Number of isolations (60 samples each examined)		Total number of positive samples
		Unwashed	Washed	
A	*S. anatum*	3	0	3
	S. bredeney	0	2	2
	S. give	3	1	4
B	*S. anatum*	14	10	24
	S. bredeney	1	2	3
	S. derby	1	0	1
	S. newport	2	1	3
C	*S. java*	4	0	4
D	*S. san diego*	1	0	1
E		0	0	0

Table 10. Median of the total aerobic count per cm.^{2} of surface of giblets held at -29° C. (-20° F.) for 0, 1, 2, 4, 8, and 12 months

Replicate	Storage period in months					
	0	1	2	4	8	12
			Gizzards			
1	310	280	113	5,100	53	440
2	2,200	3,600	1,875	10,200	8,900	25,000
3	890	1,265	965	45	1,225	7,750
4	275	100	2,390	68	328	38
5	2,450	583	440	133	8	93
6	225	3,943	6,375	158	118	293
7	268	235	248	70	680	2,120
			Hearts			
1	255	90	223	20	10	23
2	605	173	410	4,425	13	9,975
3	205	1,675	43	33	215	8,325
4	640	83	1,020	25	25	1,975
5	858	7,875	16,400	13	5	4,325
6		12,225	623	425	30	20
7		143	40	48	370	40
			Livers			
1	485	55	25	30	23	13
2	430	173	373	88	280	25,000
3	965	2,350	4,025	95	565	450
4	1,483	313	2,918	80	45	3,375
5	1,048	1,510	4,950	20	15	95
6	853	13	215	378	25	18
7	250	65	68	150	413	40

similar to those from the unfrozen samples. It can be seen in Table 11 that there are no data for the second replicate of 12 months' frozen storage or the fourth replicate of 2 months' frozen storage of gizzards and livers. Violet red bile plates

Table 11. Median of the coliform count per cm.^{2} of surface of giblets held at -29° C. (-20° F.) for 0, 1, 2, 4, 8, and 12 months

Replicate	Storage period in months					
	0	1	2	4	8	12
			Gizzards			
1	15.0	<0.1[a]	<0.1	<0.1	<0.1	<0.1
2	25.0	<0.1	<0.1	0.1	<0.0	
3	5.0	<0.1	0.2	<0.1	<0.1	15.0
4	<0.1	<0.1		<0.1	<0.1	<0.1
5	3.0	<0.1	<0.1	<0.1	<0.1	<0.1
6	0.9	<0.1	<0.1	<0.1	<0.1	<0.1
7	3.0	0.1	0.4	0.2	0.1	0.2
			Hearts			
1	10.0	<0.1	<0.1	0.2	<0.1	0.2
2	23.0	<0.1	<0.1	<0.1	<0.1	6.6
3	<0.1	<0.1	4.3	<0.1	0.2	1.6
4	<0.1	<0.1	0.1	0.1	<0.1	<0.1
5	3.0	<0.1	0.6	<0.1	<0.1	0.2
6		<0.1	<0.1	<0.1	<0.1	0.4
7		0.5	0.6	<0.1	<0.1	0.2
			Livers			
1	14.0	3.0	<0.1	0.8	1.2	0.8
2	43.0	<0.1	1.9	0.3	0.8	
3	5.0	<0.1	0.9	0.2	3.0	3.3
4	228.0	<0.1		1.3	0.1	3.3
5	3.0	0.6	0.5	0.4	0.4	0.4
6	9.9	0.4	<0.1	2.0	0.1	0.1
7	4.1	0.4	0.7	0.1	0.8	0.3

[a]No colonies detected at lowest dilution.

Table 12. Median of the enterococcal count per cm.^{2} of surface of giblets held at -29° C. (-20° F.) for 0, 1, 2, 4, 8, and 12 months

Replicate	Storage period in months					
	0	1	2	4	8	12
			Gizzards			
1	55.0	25.0	85.0	2.3	3.4	3.9
2	35.0	70.0	3.5	0.2	1.4	0.9
3	8.0	0.5	5.3	3.1	2.7	0.7
4	25.0	13.0	0.6	0.5	0.2	<0.1[a]
5	28.0	0.6	0.3	0.2	<0.1	0.6
6	9.8	40.2	0.7	0.2	0.2	1.6
7	15.9	0.2	0.2	0.8	0.5	0.5
			Hearts			
1	230.0	2.3	5.0	1.4	2.7	3.4
2	38.0	5.0	2.3	1.6	0.2	<0.5
3	<0.1	70.0	7.5	2.6	4.7	1.6
4	270.0	23.0	0.2	14.2	0.2	2.5
5	3.0	0.5	1.0	0.5	0.2	0.6
6		1.2	0.9	0.2	0.5	1.2
7		2.0	2.5	1.1	3.9	0.6
			Livers			
1	250.0	13.0	13.0	6.6	4.8	1.8
2	<0.1	0.3	15.3	4.1	39.9	13.7
3	23.0	67.5	17.6	4.4	11.7	10.3
4	448.0	12.5	1.4	19.8	1.6	1.1
5	13.0	9.0	9.5	4.9	7.6	13.4
6	5.2	1.2	0.2	1.1	0.3	0.1
7	36.9	2.3	2.8	3.3	4.0	2.1

[a]No colonies detected at lowest dilution.

from these samples showed the presence of many small colonies which precipitated the bile around the colony and reduced the dye, but otherwise did not appear as typical coliforms. Therefore the colonies on these plates were not counted. Similar to coliforms, enterococci were present at about the same level or in lower numbers during frozen storage than they were on unfrozen giblets (Table 12). It may also be observed that during frozen storage the enterococcal counts decreased more on the surface of the gizzards and hearts than on the liver surface. This also is in agreement with the results of the coliform counts.

Coagulase positive staphylococci were isolated from only three of 210 gizzards (1.4 percent), two of 210 hearts (1 percent) and seven of 210 livers (3.3 percent). Isolations were made after frozen storage for one month (two gizzards and two hearts), two months (two livers), and one year (one gizzard and five livers). The numbers of coagulase positive staphylococci isolated ranged from 50 to 700 organisms per giblet surface (as on the fresh samples). No salmonellas were detected on any of the giblet surfaces.

C. Types of Microorganisms Commonly Associated with Processed Giblets

Genera of microorganisms isolated from the surface of turkey giblets are presented in Table 13. It can be seen from

Table 13. Genera of microorganisms isolated from the surface of turkey giblets

Genus	Giblet surface		Drainage water	Total number of cultures
	Before washing	After washing		
Bacteria				
Achromobacter	7	3		10
Aerobacter		8		8
Alcaligenes	3	8		11
Arthrobacter	3			3
Bacillus		1		1
Brevibacterium	10	14		24
Corynebacterium	31	26		57
Escherichia	12	3	4	19
Lactobacillus	11	3		14
Micrococcus	16	10		26
Proteus		1		1
Pseudomonas	7	41	2	50
Sarcina	15	18	1	34
Staphylococcus	2			2
Streptococcus	15	3		18
Unclassified Gram negative bacteria				11
Unclassified Gram positive bacteria				4
Yeasts				
Candida		2		2
Rhodotorula				1
Trichosporon				1
Total				296

this table that Corynebacterium was the most common genus of bacteria isolated. Strains of Pseudomonas, Sarcina, and Micrococcus were also recovered more often than the other genera listed. Of the 296 organisms isolated, 58 had a yellow pigment and one (Rhodotorula sp.) was red. In a study of growth limits, 95 (32 percent) were able to grow at 45° C. (113° F.) in 5 days or less and 72 (24 percent) produced visible growth at 2-3° C. (35.6-37.4° F.) in 10 days or less.

V. DISCUSSION

A. Importance of Washing in Reducing Bacterial Numbers on Giblet Surfaces

1. Total aerobes

Various workers (Goresline et al., 1951; Gunderson et al., 1954; Drewniak et al., 1954; Walker and Ayres, 1956, 1959; May, 1962) observed the usual number of bacteria per cm.^{2} of poultry surface to range from 1,500 to 44,000. Other researchers (Shrimpton and Stevens, 1961; Wilkerson et al., 1961; Farrell and Barnes, 1964) have reported that sometimes the average bacterial load exceeded 100,000 per cm.^{2}. Bacterial populations on the visceral cavity surface varied from 200 to 12,000 per cm.^{2} (Goresline et al., 1951; Walker and Ayres, 1956, 1959). The skin surface of poultry during processing generally harbors more microorganisms than the surface of the visceral cavity and a giblet surface bears less microorganisms than the visceral cavity surface. Since Essary and Howes (1960) recovered only 0-2 organisms from chicken liver tissue when the surface of the bird was disinfected with 70 percent ethyl alcohol before opening, it may be assumed that contamination of the surface of giblets is a direct result of processing. One objective of processing, therefore, would be to keep bacterial contamination on giblet surfaces to a minimum during preparation.

In processing Plants A, B, C, and D, washing giblets provided at least a 90 percent reduction in the number of total aerobic bacteria present on the surface of giblets. Washing was equally effective in these plants and the decrease in microbial populations did not depend upon the type of washer used. These findings are similar to results obtained from washing whole chicken or turkey carcasses (Gunderson et al., 1946; Wilkerson et al., 1961; May, 1961; Kotula et al., 1962; Farrell and Barnes, 1964). The reduction in the total number of bacteria was most likely due to the dilution and removal of the bacteria by the flowing water.

In Plant E, no apparent reduction in the total number of aerobes occurred. These findings in Plant E were subjected to statistical analysis (Table 16). An analysis of variance indicated that there were no significant differences in the total aerobic population on samples from different plant visits, from livers or gizzards, or resulting from washing giblets. It should be recalled that in this plant livers were washed in a tumbler washer and gizzards were washed by hand.

In order to more fully determine whether washing had no effect on reducing the total number of organisms present, or if giblets were recontaminated after washing, an investigation was performed in which Serratia marcescens was used as an indicator. Numbers of S. marcescens were greatly reduced by washing, but this organism was recovered

from uninoculated livers passing through the washer 45 minutes after the inoculated giblets had been washed. Contamination of uninoculated livers also occurred during experiments conducted in the laboratory. These results indicated that the rate of change of water used in the tumbler washer in Plant E did not provide sufficient volume to result in dilution and removal of a significant number of total aerobes.

2. Fecal indicator organisms

Numbers of enterococci and coliforms present on giblet surfaces were reduced regardless of the type of equipment used for washing. Each of these types of organisms generally comprised about 1 to 2 percent of the total population before and after washing, except in Plant E where they decreased from approximately 1 to 2.5 percent on unwashed giblets to 0.1 percent or less on washed giblets. The ratio of coliforms to total aerobes was similar to that obtained by other workers (Walker and Ayres, 1956, 1959) from the surface of the visceral cavity of chickens and turkeys, but higher than the ratio found for the skin surface of poultry. Gunderson et al. (1954) observed that coliforms ranged from about 0.5 percent to about 2 percent of the total aerobic count on the skin surface of chickens. The ratio of enterococci to total aerobes was higher on giblet surfaces than that obtained by Wilkerson et al. (1961) for the skin surface of turkeys.

These findings imply that giblets and visceral cavity surfaces may possibly have been more subject to fecal contamination than was the skin of the eviscerated bird.

In Plant E, numbers of enterococci decreased from approximately 2.5 percent to 0.1 percent and the coliforms were reduced from about 1 percent to less than 0.1 percent of the total aerobic population. These results indicated that the fecal indicator organisms, but not the total number of aerobes, were diluted and removed from the surface of giblets washed in Plant E. In agreement with the findings of the study of inoculated livers, this selective reduction of fecal indicators suggested that the giblet surface was recontaminated during washing. The counts of fecal indicators also demonstrated that although hand washing of the gizzards reduced the counts of these organisms, it did not reduce the total bacterial load. Gizzards, similar to livers, were probably recontaminated during or after washing.

3. Salmonellae and coagulase positive staphylococci

Although no Salmonellae isolations were made from the surface of washed giblets in three of the processing plants, washing should not be relied upon to render giblets free of these pathogens. However, washing did reduce the incidence of these bacteria in the other two plants and provided a decrease in population of total aerobes and fecal indicators.

While S. typhimurium has been reported to be the most common human Salmonella pathogen isolated from poultry and poultry products (Edwards, 1939; Edwards et al., 1948; Browne, 1949; Felsenfeld et al., 1950; Galton et al., 1955; Brobst et al., 1958; Wilson et al., 1961; Bigland, 1963; Dixon and Pooley, 1962), it was not recovered from the surface of turkey giblets. S. give, S. java, and S. san diego have not been isolated frequently from poultry. One or more of the other Salmonella species isolated, S. anatum, S. bredeney, S. derby, and S. newport, have been recovered from poultry by other workers (Edwards, 1939; Felsenfeld et al., 1950; Galton et al., 1955; Tailyour and Avery, 1960; Wilson et al., 1961).

Although no salmonellas were recovered in Plant E, it was possible that these bacteria were present in this processing plant either in very low numbers or were not present on the days that samples were collected. The latter supposition was based on the fact that all four isolations from Plant C were made on one day from unwashed giblets sampled at the same time and from the observation that only one isolation from 120 samples was made at Plant D. These infrequent isolations indicate the difficulty in isolating salmonellas, possibly because of their poor growth in competition with other microorganisms or their low incidence on giblet surfaces. If samples were collected at frequent intervals over a long period of time, results indicate that Salmonellae might

be present on one percent or more of all giblets processed. The large number of recoveries of salmonellas (35 of 120 samples) from Plant B was probably due to infected flocks entering the plant rather than to faulty sanitation practices or workers. The sanitation routine in this plant was satisfactory and the plant appeared to employ the highest ratio of supervisory personnel to line workers of any plant visited.

As in the case of salmonellas, washing did not result in complete removal of coagulase positive staphylococci from giblet surfaces. These bacteria were recovered only three times from washed giblets in contrast to 66 isolations from the surface of unwashed giblets. The observation was made that coagulase positive staphylococci were detected more frequently in Plant A than in any other processing plant. Although these organisms could have been present in higher numbers in this plant than in any other plant, it is more likely that the increase in the size of the inoculum was responsible for the detection of more coagulase positive staphylococci in this plant.

A maximum of only 700 coagulase positive staphylococci were detected on giblet surfaces in contrast to the million or more organisms believed to be required to produce food poisoning. Salmonellae did not seem to present any serious danger of transmission by turkey giblets. It must be borne in mind that although these two food poisoning bacteria were

found infrequently on the surface of washed giblets and usually would not grow well in competition with other microorganisms, their presence on giblets still constitutes a potential hazard.

B. Comparison of Giblets for Rate of Decrease in Numbers of Bacteria During Frozen Storage

1. Total aerobes

There was a wide variation in the counts of total aerobes during storage at -29° C. (-20° F.). With eight replicates, the bacterial count at 12 months was less than one tenth the initial count. No such differences existed between the initial count and the count at 12 months in seven of the replicates. The bacterial loads with six replicates at 12 months were greater than the initial population by a factor of ten or more times. Even when the counts were lower at the end of the storage period, some of the samples at intermediate periods had higher counts than did the initial samples. This fluctuation in numbers did not appear to be associated with specific replicates, with individual storage periods, or with the giblet sampled. Other workers (Sulzbacher, 1950; Hartsell, 1951; Wilkerson *et al.*, 1961) have also reported that total aerobic bacterial counts sometimes increased during frozen storage.

Sulzbacher (1950) explained that the increased count

could represent a combination of experimental variables and growth that occurred during the cooling period after the samples were placed in the freezer. Another possibility is that some clumps of bacterial cells were broken up by freezing and in subsequent plating appeared as individual colonies rather than as one colony. The variation in results may also have been due to some difference in thawing times and temperatures used. However, other workers have shown that the thaw time and temperature may not have been an important variable. Preliminary tests by Ross and Thatcher (1958) indicated that partial thawing of frozen products at 5° C. (41° F.) for 10 to 12 hours did not significantly influence the numbers of microorganisms recovered. Elliott and Straka (1964) placed frozen chicken meat in cool running water for about one hour for a rapid thaw or allowed it to stand at 2° C. (35.6° F.) for about 24 hours for a slow thaw. They concluded that the rate of thawing made no difference in the subsequent growth at 2° C. (35.6° F.) of bacteria on the surface of the chicken.

2. Fecal indicator organisms

The results of studies of the survival of coliforms and enterococci on the surface of giblets are more nearly what would be expected during frozen storage. That is, with very few exceptions, the counts of the fecal indicators after one

month of frozen storage were much lower than the initial counts and the populations showed little fluctuation from the end of the first month's storage to the end of the frozen storage study at one year.

The observation that coliforms did not survive as long as did enterococci is in agreement with other reports of coliform-enterococcus ratios in poultry and poultry products (Kereluk and Gunderson, 1959; Hartman, 1960a,b; Wilkerson et al., 1961; Kraft et al., 1963).

C. Types of Microorganisms Isolated from Fresh Eviscerated Poultry

A comparison of the types of microorganisms isolated from turkey giblets with those organisms isolated by other workers (Gunderson et al., 1947; Ayres et al., 1950; Stadelman et al., 1957; Barnes and Shrimpton, 1958; Thatcher and Leit, 1961; Barnes, 1960a,b) from fresh eviscerated poultry is presented in Table 14. Organisms which were isolated from giblets and which were not recovered from poultry carcasses include Arthrobacter, Brevibacterium, and Lactobacillus. The remaining 16 genera listed as having been recovered from giblets have also been isolated by one or more of the other researchers. It is interesting to note that Pseudomonas has been reported by all the workers listed: Achromobacter was the next most common isolate, and was

Table 14. Types of microorganisms isolated from fresh eviscerated poultry

	Investigators							
	Gunderson et al. (1947)	Ayres et al. (1950)	Stadelman et al. (1957)	Barnes and Shrimpton (1958)	Thatcher and Loit (1961)	Barnes (1960a)	Barnes (1960b)	This study
Bacteria								
Achromobacter	+	+		+	+	+	+	+
Aerobacter	+	+			+			+
Alcaligenes	+	+			+			+
Arthrobacter								+
Bacillus	+	+						+
Brevibacterium								+
Citrobacter (Paracolobactrum)	+							
Corynebacterium	+			+		+	+	+
Cytophaga								
Escherichia	+	+			+			+
Flavobacterium	+	+	+	+	+			
Gaffkya	+							
Hemophilus								
Lactobacillus								+
Microbacterium	+							
Micrococcus	+	+						+
Neisseria	+							
Proteus	+	+						+
Pseudomonas	+	+	+	+	+	+	+	+
Salmonella (Eberthella)	+	+						+
Sarcina	+	+						+
Staphylococcus	+	+						+
Streptococcus	+	+					+	+

Table 14. (Continued)

	Investigators							
	Gunderson et al. (1947)	Ayres et al. (1950)	Stadelman et al. (1957)	Barnes and Shrimpton (1958)	Thatcher and Loit (1961)	Barnes (1960a)	Barnes (1960b)	This study
Molds								
Actinomyces								
Geotrieum (Oidium)	+							
Oospora		+						
Penicillium		+						
Streptomyces		+						
Yeasts								
Candida								+
Cryptococcus		+						
Rhodotorula		+						+
Saccharomyes			+					
Trichospóron		+						+
Torula	+							

recovered in seven other investigations. Both Escherichia and Flavobacterium were isolated in five studies, although Flavobacterium was not found on the giblet surface.

In general, the microbial genera were found in the same proportions on unwashed as on washed giblets. However, there appeared to be a decrease in Escherichia, Lactobacillus, and Streptococcus due to washing and an increase in the number of Aerobacter and Pseudomonas recoveries. Since species of the genus Pseudomonas have been very infrequently isolated from the gut of chickens being eviscerated (Barnes, 1960b) and have not been recovered from human feces (Ingraham, 1958), these bacteria probably originate from sources other than the gut. Barnes (1960b) believed that Pseudomonas entered the processing plant in small numbers in the water supplies, feathers and feet of the birds, ice slush, and equipment, and then multiplied readily on dirty surfaces, in the ice slush tanks, and on the chickens.

As shown in the Results section, Corynebacterium was isolated more often than any other genus and comprised about 25 percent of the total population. Together with Achromobacter, this genus was found in very large numbers in the feathers and on the feet of chickens entering a processing plant (Barnes, 1960a,b). Assuming that the flora on the feathers and feet of turkeys is similar to that found on chickens,

Corynebacterium conceivably could comprise much of the contamination on giblets.

VI. CONCLUSIONS

The following conclusions were made in regard to the processing of turkey giblets.

1. By flowing fresh water over the surface of giblets, the total bacterial population undergoes a 90 percent reduction. This reduction is due to the dilution and removal of the microorganisms by the water.
2. With fresh water, the type of washing procedure used is not important in removing bacteria. However, when dirty water is allowed to remain in a washer, bacteria are transferred from contaminated to uncontaminated giblets.
3. Numbers of enterococci and coliforms present on giblet surfaces are reduced regardless of type of equipment used for washing.
4. Washing does not result in giblet surfaces free of coagulase positive staphylococci or salmonellas.

The following conclusions were reached in regard to the frozen storage of turkey giblets.

1. There is no appreciable difference in bacterial loads on different kinds of giblets.
2. The counts of the fecal indicators after one month of frozen storage are much lower than the initial counts and the populations show little fluctuation

from the end of the first month of storage to the end of one year.

VII. SUMMARY

Determinations were made of the number and types of bacteria associated with turkey giblets during commercial processing. Swab samples were obtained from the surface of giblets being processed in five federally inspected processing plants. These samples were examined for total aerobic bacteria, coliforms, enterococci, coagulase positive staphylococci, and salmonellas. Bacteria isolated from the giblets were identified to genus. Giblets other than those examined in the processing plant were frozen in the laboratory for studies of the effect of length of time in frozen storage on survival of bacteria on the surface of these giblets.

In all processing plants the total number of aerobes present on giblets was not great and organisms having public health significance were present in low numbers or not at all. In four of the five plants visited, washing with water served to decrease the numbers of bacteria present on the giblet surface by at least 90 percent. In the fifth plant, which was one of two plants that employed tumbler washers, washing did not reduce the total number of organisms present.

A study with the indicator organism Serratia marcescens was conducted in the fifth processing plant and in the laboratory to determine why the tumbler washer in this plant was not effective in reducing microbial populations. This investigation indicated that numbers of S. marcescens were greatly

reduced after washing. Uninoculated livers were then contaminated by the inoculum in the water for as long as 45 minutes after the inoculated livers had been washed.

Seven species of Salmonella were recovered from giblet surfaces. S. anatum was isolated more frequently than any of the other types. There were fewer recoveries of salmonellas from the surface of washed giblets than from unwashed giblets. Coagulase positive staphylococci were also found on fewer of the washed giblets than the unwashed giblets.

In general, the microbial genera were found in the same proportions on the unwashed as on the washed giblets. Corynebacterium was isolated from the surface of turkey giblets more often than any other genus. Sarcina species were also common contaminants of giblet surfaces. Pseudomonas species were a small portion of the microorganisms on the surface of unwashed giblets, but were the predominant organisms on the surface of washed giblets.

The effect of frozen storage on the total aerobic bacterial population was not consistent. With some of the samples examined, the number of bacteria decreased. In other instances, the counts at the end of one year of frozen storage greatly exceeded the counts of the unfrozen samples. Loads of coliforms and fecal streptococci were lower at the end of one month of frozen storage than they were on the giblets before freezing. There was little difference in the populations of

fecal indicators from the end of the first month to the termination of frozen storage after one year. Enterococci were usually isolated in higher numbers than coliforms from the surface of frozen giblets. The microbial population was not influenced by the type of giblet.

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X. APPENDIX

Table 15. Analysis of variance of total count

Source of variation	Degrees of freedom	Sum of squares	Mean square
Visits	4	3.91	0.98
Treatments	3	0.78	0.26
Giblet (G)	(1)	0.00	0.00
Station (S)	(1)	0.75	0.75
G x S	(1)	0.02	0.02
Experimental error	12	14.67	1.22
Total	19	19.36	

Table 16. Incidence of total aerobic bacteria on turkey giblets from five processing plants (numbers indicate the percent of samples distributed within a given range of counts)

Processing plant	Number of samples before and after washing	Before washing				After washing			
		0-10	10-100	100-1,000	1,000-10,000	0-10	10-100	100-1,000	1,000-10,000
A	60	0.0	23.7	47.5	27.1	18.3	75.0	5.0	1.7
B	100	8.0	43.0	42.0	7.0	15.0	69.0	15.0	1.0
C	100	10.1	54.6	31.3	4.0	58.6	41.4	0.0	0.0
D	100	9.0	41.0	40.0	10.0	18.0	54.0	18.0	1.0
E									
Livers only	50		18.0	62.0	20.0	2.0	24.0	54.0	20.0
Gizzards only	50		12.0	68.0	20.0	0.0	28.6	53.1	18.3
Giblets	100	0.0	15.0	65.0	20.0	1.0	26.3	53.5	19.2

Table 17. Incidence of coliform bacteria on turkey giblets from five processing plants (numbers indicate the percent of samples distributed within a given range of counts)

Processing plant	Number of samples before and after washing	Before washing			After washing		
		0-10	10-100	100-1,000	0-10	10-100	100-1,000
A	60	40.0	60.0	0.0	100.0		
B	100	50.0	45.0	5.0	95.0	5.0	
C	100	92.9	7.1	0.0	96.9	1.1	
D	100	96.0	4.0	0.0	90.0	10.0	
E							
Livers only	50	63.3	36.7	0.0	100.0		
Gizzards only	50	96.0	4.0	0.0	100.0		

Table 18. Incidence of enterococci on turkey giblets from five processing plants (numbers indicate the percent of samples distributed within a given range of counts)

Processing plant	Number of samples before and after washing	Before washing			After washing		
		0-10	10-100	100-1,000	0-10	10-100	100-1,000
A	60	15.0	60.0	25.0	100.0	0.0	
B	100	43.2	55.8	1.0	91.0	9.0	
C	100	85.9	14.1	0.0	100.0	0.0	
D	100	84.5	15.5	0.0	100.0	0.0	
E							
Livers only	50	42.0	56.0	2.0	98.0	2.0	
Gizzards only	50	59.2	40.8	0.0	94.0	6.0	
Giblets	100	51.0	49.0	0.0	96.0	4.0	