

A REVIEW OF THE MICROBIOLOGY OF FRESH MEAT

by 6408

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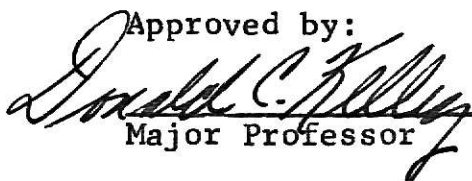
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INTRODUCTION

SYNOPSIS

This report on "A Review of the Microbiology of Fresh Meat" deals with the microbiological implications of fresh meat beginning with slaughtering operations and extending through retail packaging. The report is divided into three general parts. Under the heading of "Contamination During Slaughter and Dressing" sources of both superficial and deep tissue contamination to the carcass are discussed. In the second section the effects of chilling, storage and shipping on the microbial flora are reviewed. The microbial involvement during cutting, comminuting, packaging and freezing is related in the final section.

FOREWORD

Toward the latter part of the 19th Century, as the "germ theory" of disease rapidly gained a firm footing in medical opinion, bacteriology began to merge as a definite science. The question of bacteria in meats was raised and it soon became evident that meat favored the multiplication of many kinds of microorganisms reaching it from various sources.

Since that time our understanding and control of these phenomenon has been greatly extended due to a number of workers,

especially in Great Britain, Australia, New Zealand, Germany, and the United States, who have conducted studies on the incidence, types, sources and significance of microorganisms in meat.

Meat and meat products often contain high numbers of microbial cells compared to some other foods even when they are organoleptically satisfactory. This is largely because meat is an almost ideal medium for bacterial growth.

Meat that is excessively contaminated with microorganisms is undesirable from several points of view, namely public health aspects, storage quality, and general esthetic principles. Thus, the basic purpose for a thorough knowledge of the microbiology of meat appears to be averting spoilage and preventing meat-borne infection, by reducing to a minimum the opportunities for microorganisms, particularly the pathogens, to gain access to meat and to proliferate therein. Organisms concerned with meat spoilage and meat-borne diseases may enter the product during its handling or may be in the tissues of the live animal as offered for slaughter.

PART ONE: MICROBIAL CONTAMINATION DURING SLAUGHTERING AND DRESSING

Superficial Contamination

Sanitary handling of meat begins with the slaughter and dressing operations. The normal healthy animal as presented

for slaughter is heavily contaminated with microorganisms both on the outside surface as well as throughout the intestinal tract. In view of these conditions, it is not feasible to completely prevent contamination, however, effective control over sanitary dressing procedures is vital to minimize the transfer of microorganisms to the meat. It has been shown by Scott, 1931; Haines, 1931a, 1933a,b; Empey et al., 1934, 1939a,b; Jensen and Hess, 1941; Ayres, 1955, 1956; Patterson, 1967, 1968a,b, 1969; and Stringer et al., 1969, that the initial invasion of microorganisms begins with the slaughtering operations.

During the slaughter and dressing operations flora that come in contact with the superficial surface of the carcass are derived from the air, hide, gastrointestinal contents, hand tools and personnel. Scarafondi (1957) has summarized the work of Empey and Scott (1939a) who made a thorough study of sources of contamination of beef carcasses during slaughtering operations. The main sources were as follows:

- (1) dirt and skins of animals.....(approximately 33%);
- (2) air (atmosphere) of the
slaughtering department.....(approximately 5%);
- (3) viscera contents-normal
conditions.....(approximately 3%);
- (4) halving, quartering and packing
of carcasses.....(approximately 2%);

(5) storage and transport.....(50% or over);

(6) equipment and personnel.....(approximately 3%).

Since the fundamental principles of hygiene demand that the slaughtering of animals for human consumption be carried out with the least amount of contamination possible, every precaution must be made to prevent or to remove such contamination. Therefore, the principle objective of the dressing procedures should be to remove or clean the hide or skin and to remove the gastrointestinal tract and other internal organs with a minimum of contamination to the meat.

To emphasize the importance of proper dressing techniques, Empey and Scott (1939a) found that by the introduction of hygienic methods of dressing the extent of contamination was reduced to approximately 5 percent of its former level (from a mean value of 130,000 down to 7,000 organisms per sq. cm.) and obtained an increase storage life of from three to eight days when stored at -1°C (30.2°F).

Skin or Hide

The protective covering of skin or hide is covered with hair, manure, and dirt, and is in itself a source of objectionable contamination from the esthetic sense. But of even more significance, these potential contaminants contain a wide variety and exceedingly large number of microorganisms.

It has been pointed out by a number of workers (Empey and Vickery, 1933; Empey et al., 1934; Haines, 1937; Empey and Scott, 1939a,b; Mallman et al., 1940), that the flora which ultimately develops on the meat comes principally from soil organisms associated with the hoofs and hides of the animals.

Soils contain large numbers of microorganisms, including Pseudomonas, and differ greatly from season to season and area to area. Good fertile soil may contain from 100,000 to 0.5 billion or more live microorganisms of all types per gram (Umbreit 1962).

The bacterial flora of the skin is composed of two basic types, (1) residents and (2) transients (Price 1938). Transient bacteria are collected from extraneous sources and vary tremendously in number and species depending on the animal's environment. They are comparatively easy to remove or kill and may include both saprophytic and pathogenic species. The resident bacteria are firmly attached to the skin and include some pathogenic organisms. Staphylococci such as Staph. epidermis and Staph. pyogenes occur commonly as do other micrococci (Carter 1967).

Empey and Scott (1939a) reported from 100,000 to 31,000,000 total bacteria (mesophilic and psychrophilic) per sq. cm. of unwashed hide surface, with mean numbers of 3,300,000 per sq. cm.

The mean count for the "low temperature type" organisms (ones capable of growing at -1°C (30.2°F) was 15,000 per sq. cm., the mean counts for yeasts and molds being 580 per sq. cm. The hide microflora, to an extent, was the same as the microflora of soils on which the cattle grazed. Variations in counts were found from the various areas of hide from the same animal, probably due to contaminations acquired through contact with the soil.

Seasonal variations were also noted for the incidence of low temperature type organisms present in the soil, on the hide and among carcass contamination. The incidence increased during the colder seasons and decreased during the warmer months. It was surmised from these findings that meat prepared in tropical zones would carry a smaller percentage of low temperature bacteria, and therefore would be less susceptible to spoilage during storage.

Jensen and Hess (1941) reported from 100,000 to 1.5 trillion aerobes and from 10,000 to 2 trillion anaerobes on 2 sq. in. of neck skin of swine in the area where the jugular vein is cut. The mean count for yeasts and molds was 850 per sq. cm.

Gastrointestinal Tract

The importance of precaution during evisceration procedures to avoid rupture of the intestine or stomach has been emphasized

(Patterson, 1969). In the United States, meat inspection procedures require that the rectum and esophagus of beef carcasses be tied to prevent leakage of contents. When done properly, very little contamination should occur as proper tying has proven successful in reducing bacterial contamination from the gastrointestinal tract (Empey and Scott, 1939a; Patterson, 1969; Childers and Keahey, 1970).

The kinds and numbers of microorganisms present in the gastrointestinal tract vary greatly. The flora is influenced by the digestive habits of the animal (Ayres 1955), age, geographical location, nutrition and climate (Carter 1967).

Not only are microorganisms ingested with feed and water, but their propagation and growth are very significant parts of the digestive process, so much so that much of the fecal mass is actually a concentration of microorganisms in astronomically large numbers (Ayres 1955).

Empey and Scott (1939a) found the mean count of rumen content on a dry weight basis was 53,000,000 bacteria, 180,000 yeasts and 1,600 molds per gram.

In the lower small intestine bacteria grew abundantly so that the microbial population increased progressively until, in the colon and rectum, large numbers were present (Ayres 1956).

From five observations made of freshly voided bovine feces, Empey and Scott (1939a) reported mean counts of 90,000,000 bacteria, 200,000 yeasts and 60,000 molds per gram on a dry weight basis. Thornton (1962) related that fresh bovine feces may contain as high as 150,000,000 bacteria per ounce.

Some genera of bacteria that can be expected to occur normally in the large intestine of food animals include: Fecal streptococci; Escherichia coli, Klebsiella, Aerobacter, Pseudomonas spp.; Proteus spp.; enterococci; staphylococci; Clostridium welchi, Cl. septicum; Bacteroides spp.; spirochetes; lactobacilli (Smith and Crabb, 1961; Carter, 1967).

In addition to spoilage organisms being present in feces, a more important hazard is the presence of potential human pathogens. Of these probably the most important is the genus Salmonella. Many cases of human food poisoning each year point to meat and meat products as important vehicles of infection. Some of the contamination undoubtedly originates during the dressing operations (Weissman and Carpenter, 1969).

Knives, Saws and Other Cutting Tools

During the slaughter and dressing operations, knives, steels, saws, cleavers and other types of equipment rapidly become contaminated.

Empey and Scott (1939a) reported the population of bacteria on knives used for incising pieces of hide ranged between 40,000 and 80,000 per blade.

Jensen and Hess (1941) considered the stick knife as a very important avenue for the introduction of organisms into the tissues. They theorized that on the basis of the amount of serum or liquid blood in a 250 pound hog, a knife blade introducing only 50,000 bacteria could infect the circulating blood with many bacteria in the short span of life after sticking.

In addition to the contamination acquired through contact with the skin or hair, knives may also become contaminated through contact with the steel, clothing of the worker, or pouches used for holding knives. Up to one billion organisms per gram have been found in the material scraped from the inner surface of pouches (Empey and Scott, 1939a). Saws and cleavers which had been in use for several hours showed populations in the order of 150,000,000 per gram of material adhering to the surfaces. They were able to demonstrate that these surfaces were responsible for varying degrees of transfer during their use.

Brune and Oldham (1970) noted that the total bacterial count on the knife and steel used by a swine eviscerator

doubled after eviscerating just five hog carcasses (from 600 to 1200 per sq. cm. mean counts).

Gustin and Siddiqi (1970) noted that the areas most contaminated on freshly dressed hog carcasses were the areas where the workmens' knives and saws came in contact, namely the mid-line and chine area.

Proper sanitation of equipment during operations has been shown to reduce contamination of the carcass. Patterson (1969) stressed the importance of conveniently located handwashing facilities and sterilizers for hand tools for reducing contamination. Galton et al., (1954b) demonstrated a marked reduction of Salmonellae on carcasses following thorough cleaning of work areas and equipment.

Workers and Inspectors

Education of the workers is the first requirement for any kind of a successful sanitation program. Probably the most important factor in the dressing operation is cleanliness of the techniques used by workers (Patterson, 1969).

The hands, arms and clothing of workmen receive their major contamination through contact with the hair of the hides. Clothing of workers engaged in the skinning operation acquired levels as high as 3 billion per gram of material that could be scraped from their clothing after skinning approximately 100

carcasses during a 6-hour period (Empey and Scott, 1939a). The population acquired on the hand of a workman during the handling of the hair on about 100 sq. cm. of hide reached a total count of 2,000,000.

During the dressing operations, workmen, in making the necessary incisions, often come in contact with the outer surface of the carcass as well as the visceral cavity. Meat inspectors, as well, are required to touch edible parts of the carcass during the course of inspection and bacterial transfer from one carcass to another undoubtedly can occur as a result of these manipulations. The effect that present inspection procedures have on carcass contamination has not been studied.

In addition there is the possibility of directly transferring human pathogens (Horwood and Minch, 1951; Patterson, 1969).

Air

The air and general environment of the slaughtering department is another very important avenue for carcass contamination.

The source of air-borne flora in this area is partly derived from the milling and thrashing of animals while they are on the killing rail or in the knocking pens (Haines, 1933), and partly from the atmosphere surrounding the plant (Empey and Scott, 1939a).

Empey and Scott (1939a) reported that the microbial deposits from air of slaughter floors of about 30 bacteria and about 2 molds per sq. cm. per hour. The following types of microorganisms have been found in the air of slaughtering floors: Staphylococcus, Micrococcus, Bacillus, Pseudomonas, Achromobacter, Flavobacteria, Azobacter, Proteus, Aerobacter, coliforms and molds (Haines, 1933a).

Empey and Scott (1939a) believed the drying power of the atmosphere had some effect upon the nature and extent of the air-borne microflora. They found yeasts and molds, which are more resistant to desiccation than bacteria, usually present in relatively higher percentages in the air-borne flora, than in the surface soils in the vicinity of the plants or on the hides of the animals. They also found that the incidence of yeasts on dressed beef sides was significantly greater than on the hide, and were unable to offer an explanation for this other than the drying power of the air.

Water

Water that is used in packing plants in the United States should not contribute many microorganisms. Potable water used in packing plants must be obtained from an approved source, sampled regularly, and the approved supply cannot be

cross-connected with a non-approved supply. (Manual of Meat Inspection Procedures, United States Department of Agriculture).

Apparently practices differ throughout the world with respect to routine washing of carcasses during dressing operations. Haines (1933a) and Empey and Scott (1939a) described the use of wash cloths for wiping the carcass at various stages of dressing in order to facilitate the removal of blood and excess moisture. Although quite effective in removing gross contamination, they were found to become heavily contaminated. Bacterial populations ranging from 40,000 to 100,000 per sq. cm. were found on these cloths (Empey and Scott, 1939a).

Haines (1933a) reported that the water used to rinse the cloths between carcasses contained as many as 130,000,000 organisms per ml. Empey and Scott (1939a) found counts as high as 350,000 per ml. When sterile cloths were used on each carcass microbial contamination could be reduced by as much as 50 percent (Empey and Scott, 1939a). The Joint FOA/WHO Expert Committee on Meat Hygiene in 1955 recommended that..."On no account should the use of dirty wiping cloths be permitted. When wiping cloths are employed they should be scrupulously clean and preferably used on only one carcass before being rewashed and sterilized." (Meat Hygiene, 1955 WHO Monograph Series No. 33, Geneva). The use of wiping cloths has been

prohibited in Northern Ireland since 1965 (Patterson, 1969).

Intermediate wash points have been recommended as quite effective when located at suitable points along the dressing line (Patterson, 1969). In the United States a wash area is used for hog carcasses just prior to head dropping operations, and is apparently very effective (Ockerman, 1969). A similar wash area is used on sheep and lamb carcasses, but its effectiveness has not been studied. It is required that washing of beef carcasses be deferred until bruises have been removed and inspection has been accomplished.

Another source of contamination can take place during washing operations if water from the carcass being washed splashes on adjacent carcasses (Patterson, 1969). Contamination can also take place from water splashing from the floor onto the carcass (Empey and Scott, 1939a).

The use of free chlorine up to 20 ppm in all water used on the slaughtering floor has been shown to cause a reduction in bacterial counts on carcasses (Patterson, 1969). The use of hot water up to 96° C (204.8° F) in the final wash was another means of lowering the counts on carcasses. On the other hand, the effect of hot water on fecal streptococci was not significant, possibly because of the greater heat resistance of these organisms (Patterson, 1969).

Childers and Keahey (1970) observed that washing the carcass after evisceration is probably responsible for the greatest spread of contamination.

Shrouds

Apparently shrouds can be an important source of contamination to the carcass. Jensen (1954) stated that microbial growth is induced at the interface of textile and water film and to a small extent on the meat itself. Stringer et al., (1969) reported that the microbial counts taken on dry shrouds and also the water in which the shrouds had been soaked revealed that shrouds contain a large number of bacteria. The mean count per sq. in. of dry shroud surface was 27,550. After the shrouds had been soaked, the mean water count per ml. was 66,100. Apparently no tests have been made to determine if shrouds contain the same flora of microorganisms as found on meat.

There is a possibility that shrouds may become sour or moldy before being used since drying following laundry is not required. No studies have been conducted in this area.

A salt solution not exceeding 20 percent salometer strength or a sodium hypochlorite solution not exceeding 20 ppm may be used on shrouds prior to their application to dressed carcasses of any species. Acetic acid solution not exceeding 1 percent may be used for wetting shrouds prior to their application to

dressed sheep carcasses (Manual of Meat Inspection Procedures of the United States Department of Agriculture, 1968). No work has been done to study the effect these chemicals may have on microbial levels on shrouds.

Scald Water

Use of the scald tank is restricted to hogs and ordinarily the water in the tank is maintained at a temperature of 60° C (140° F) (DeBeukelaer and Wang, 1955).

Few studies are available to indicate the role of the scald water as a contaminating medium. Ayres (1955) postulated that since huge numbers of microorganisms find their way into this water during the course of a day's operation, scald water may be an important avenue for microbial contamination.

Apparently the temperature of the scald water has a lethal effect on the bacteria gaining entrance into it. Ockerman (1969) reported that the scalding of carcasses had the most significant effect upon reducing the surface microbial level by removing the hair, dirt and scurf and destroying the microflora with which it came into contact. He reported the microbial "kill" to be dependent upon a time-temperature contact with the microbes. Brune and Oldham (1970) observed that the scald water in their survey had a mean total bacterial count of 17,600 per ml. Galton et al., (1954b) in their study on the incidence of

Salmonella in swine have shown that swab cultures from the sides of hogs being lifted from the scalding vat were free from Salmonella. Cultures from the vat water were also negative for Salmonella.

Effects of Successive Steps in the Dressing Operations on Superficial Contamination

Few studies on microbial contamination of meat carcasses have included a step-by-step microbiological examination of the slaughtering and dressing procedures, nor has there been a general comparison made of the "bed" and the "on-the-rail" type of beef dressing except for the small survey made in Northern Ireland (Patterson, 1968a).

Ockerman (1969) observed that the successive slaughter steps altered the contamination of hog carcasses greatly and the scalding of carcasses had a most significant effect upon reducing the surface microbial level.

The singeing of the carcass lowered the contamination appreciably as did the rinsing prior to evisceration. Shaving the carcass had no effect upon the surface flora.

The carcass contamination was found to be at its lowest point immediately following the rinse prior to evisceration. The handling of the carcass during evisceration, splitting and

inspection doubled the surface flora from the minimum point following the rinse prior to evisceration.

It was also observed that variations in dressing techniques among individual plants can appreciably affect the contamination.

Increasing the number of carcass rinses appeared to lower the superficial contamination.

Galton et al., (1954b) observed that the dehairing machine served to inoculate the skin of swine with Salmonella. Brune and Oldham (1970) experienced a similar observation using coliforms as an indicator.

Empey and Scott (1939a) found that the transfer of microorganisms from the hide to the underlying tissues on beef carcasses begins with the first stage of skinning. Populations were found ranging between 10,000 and 100,000 per sq. cm. on the superficial tissues of the carcass after skinning. Numbers of organisms in the tissues were highest in the region below the initial incision through the hide and lowest in the areas that were further removed from this region. Further transfer of microorganisms occurred when parts of the hide that had been separated came in contact with the carcass. Such areas of direct contact may receive contamination equivalent to one-third that of an equal area of hide.

From samples taken in a Northern Ireland abattoir, Patterson (1969) observed a high incidence of carcass contamination due to both contact with the hide directly and also indirectly by transfer via hands, clothing and equipment.

Areas of Carcass Studied for Contamination

Different workers have used different techniques and areas when sampling carcasses. Empey and Scott (1939a) sampled from the following areas of beef carcasses: (1) aitch bone, (2) neck on both vertebral and ventral sides of the jugular furrow, (3) brisket, and (4) connective tissue and fat covering the rump, loin, rib and shoulder areas. They reported counts ranging from 6,400 to 830,000 per sq. cm. with a mean count of 97,000 per sq. cm.

Patterson (1968a) studied the spread of contamination on beef carcasses on both "on-the-rail" and "bed" type dressing. A 16 sq. cm. area at seven sites on each half carcass was sampled. The sites sampled were: (1) hindleg, (2) rump, (3) flank, (4) sirloin, (5) brisket, (6) foreleg and (7) neck. Carcasses dressed "on-the-rail" had significantly higher levels of contamination on the brisket compared with all other sites except the rump (mean bacterial counts per sq. cm. were 4,670 and 1,320 respectively). The rump had a significantly higher level of contamination than all other sites. The hindleg,

foreleg and sirloin sites did not differ significantly from each other. There was no significant difference between the left and right sides. From carcasses dressed on the "bed" contamination appeared to be more evenly spread over the carcass. The brisket, sirloin and rump areas were significantly more contaminated than the other areas, but not significantly different from each other (mean bacterial counts per sq. cm. were 1,620, 1,350 and 1,030 respectively).

Of the sites sampled on the carcass, three (rump, sirloin and brisket) were fatty tissue, whereas the remainder were largely muscle. The fatty sites were significantly more heavily contaminated than the lean, on both types of dressing.

Patterson (1968a) observed contamination on sheep carcasses using the same areas on the carcass as described above for cattle carcasses. Again the most heavily contaminated area was the brisket (mean bacterial count per sq. cm. was 1,070). The crutch and neck sites were the least contaminated (1,350 and 912 per sq. cm. respectively). These results show that contamination acquired during the dressing operation is not spread evenly over the carcass, whether it is a sheep or beef carcass. The sites of heaviest contamination on the carcass varied from one plant to the next.

There is little published work on which to base standards, however, Patterson (1968a) has suggested the following advisory standards for cattle and sheep carcasses:

<u>Site sampled</u>	<u>Total count per sq. cm. incubated on nutrient agar for 3 days at 22° C</u>
Rump	3,000
Brisket	10,000
Foreleg	3,000

Under good conditions, these are fairly easily attained and offer a yardstick whereby dressing hygiene can be assessed. Patterson (1968a).

Stringer et al., (1969) made a study of the microbial contamination of five areas of the carcass immediately after dressing. These were: (1) inside of the neck, (2) chine bone area, (3) clod area, (4) lean surface above the aitch bone and (5) the outside fat cover on the round. They reported all the areas sampled were highly contaminated (mean carcass population was 51,300 per sq. in.). Moist areas such as the neck, lean surface above the aitch bone, and the clod area were the most highly contaminated. The chine and round areas were least contaminated. This may be expected since the bacteria should multiply faster in moister areas. A small seasonal variation was observed but season had very little effect on the degree of initial contamination on the carcass in this particular study.

Greater differences were noted in microbial populations among various lots of cattle.

Gustin and Siddiqi (1970) studied the total count and coliforms taken from six locations on dressed hog carcasses just prior to placing into cooler. The areas sampled were: (1) midline, (2) chine area, (3) brisket, (4) abdominal cavity, (5) thoracic cavity and (6) inside of neck area. They reported that the carcass areas most likely to sustain excessive bacterial contamination were those which the workmen's knife and saw came in contact, in particular the midline areas where total counts ranged from 7,100 to 25,600 per 25 sq. cm. with a mean count of 16,200 per 25 sq. cm. No pattern was established regarding the number of coliforms compared to the total number of bacteria.

Childers and Keahey (1970) in a study to determine the spread of Salmonella and coliform organisms during slaughter took swab samples of (1) the brisket, (2) midline, (3) pelvic fat, (4) diaphragm, (5) lumbar and cervical vertebrae, and (6) the neck. They observed that washing the carcass after evisceration is probably responsible for the greatest spread of contamination. They also reported that the pelvic fat in the bung areas was contaminated in fewer instances than other parts of the carcass following evisceration and washing. This

may be contrary to what many believe that the bung-dropping operation is one of the primary sources of contamination during evisceration. Possibly the care exercised in detaching and tying the bung is a factor in reducing contamination of the pelvic areas.

Apparently Felsenfield et al., (1950) were the first to examine for the incidence of Salmonella from specific areas of a carcass. From swine carcasses they sampled from: (1) the loin area (5 of 71 or 7.0%); (2) the rib area (5 of 72 or 7.0%); (3) the shoulder area (4 of 72 or 5.6%); and the tenderloin (4 of 50 or 8%).

Weissman and Carpenter (1969) showed that the incidence of Salmonella species from 50 hog and 50 beef carcasses from five different plants was 56% and 74%, respectively. Areas of the carcass sampled were: (1) outside forequarter, (2) inside forequarter, (3) outside hindquarter, and (4) inside hindquarter. The frequency of isolation of Salmonella from the areas of the carcass revealed that no particular half carcass or area of half carcass was more likely to be contaminated than any other area. Areas for representative sampling were (1) the cervical area of the neck (inside fore-quarter), and (2) the anal area (outside hindquarter), due to washing from the carcass in the former and fecal contamination in the later case.

Deep Tissue Contamination

It is generally agreed that the flesh of live and healthy slaughter animals is sterile or at the most contains only a few microorganisms under normal conditions (Jepsen, 1957). Many findings, however, indicate that bacteria may occasionally be found in the tissues post-mortem.

There has been a considerable and extensive controversy over the years in the literature regarding the source of microorganism that occasionally contaminates the deep tissues of the carcass. Many theories concerning the source of contamination have been investigated.

It has been postulated by some workers that the causative organisms gain access to the tissues as a result of post-mortem invasion from such sites as the intestinal tract, respiratory tract and lymph nodes. Others support the view that bacterial invasion may occur both after death as well as at the time of death (post-mortem and agonal). Another theory and one which has more or less been disproved by more recent work, was that microorganisms may be the inherent flora of the deep tissues.

Stress from handling prior to slaughter has been shown to have a tremendous effect on the bacterial load that can be isolated from the carcass (Haines, 1937; Ayres, 1955; Houthuis, 1957).

Many workers have conducted microbiological examinations on tissues taken from living and dead animals of various kinds. The results of these studies have been conflicting, some indicating that bacteria are commonly found in normal muscle tissue and others seemingly to demonstrate that such tissue is generally sterile.

One school of thought, as reviewed by Ayres (1955), largely from the early German workers around the turn of the century, contended that the organs and musculature of healthy animals were free from microorganisms, whereas other workers indicated that a few bacteria may be contaminants of animal tissues considered to be normal. Many of the early studies led to faulty conclusions owing to the use of defective bacteriological methods.

In more recent years with use of modern methods of bacteriology, the work of Jensen and Hess, 1941; Adamson, 1949; Lepovetsky et al., 1953; Weiser et al., 1954; Zender et al., 1958; Nottingham, 1960; Sharp, 1963; Ockerman et al., 1964; tends to support the theory that the flesh of live healthy slaughter animals is either sterile or at the most contains only a few bacteria under normal conditions.

Theories concerning bacterial invasion of the carcass within a relatively short time after slaughter have been investigated by many workers and some controversy still exists

concerning the portal of entry of these contaminants. The possibility that bacterial contamination can occur internally in the freshly slaughtered animal through permeability of the intestinal mucosa, respiratory tract and lymph nodes seems probable since there appears to be no residual bacteriostatic or bacteriocidal properties in the tissues of the freshly slaughtered animal. In the living animal there can be an equilibrium between invasion of the tissues and removal of the invading organisms such that the tissues of healthy animals are normally free from bacteria (Haines, 1937). Since the defense mechanisms are lost by the animal at the time of death, the momentary shock that accompanies exsanguination probably opens new avenues for bacterial invasion from such internal sites as previously mentioned (Hensen and Hess, 1941). In some species it would appear that the reticulo-endothelial system is more effective than in others, since venison, for example, can be hung for a considerable time at room temperature without undue precautions (Lawrie, 1966).

Invasion from the Intestinal Tract

It has been reported and now generally believed that bacteria can penetrate the intestinal barrier of exhausted animals and spread to other tissues (Arnold, 1928; Burn and Burket, 1938; Haines, 1937; Robinson et al., 1953). Fine et al., (1959) and

Frank et al., (1961) have established that bacteria regularly transverse the intestinal barrier in the live animal. These bacteria have been shown to be largely disposed of in the liver (Smith and Martin, 1948). The liver may not always eliminate all the bacteria from the portal circulation and some of these may then lodge in other tissues and become latent.

The mode of slaughter may also be implicated since breakdown of the intestinal mucosa has been observed in sheep which had been shot (Badaway et al., 1957). These observations explain the traditional reluctance to give animals food less than 24 hrs. pre-slaughter. Schönberg (1957) recommended that the feeding of animals should cease 8 hrs. before slaughter so as to prevent the penetration of bacteria from the intestines into the flesh via the blood and lymph.

The organisms from the intestinal tract which can be distributed to the muscles by the blood include various Streptococci, Clostridium welchii, and Salmonella spp. (Medrek and Barnes, 1962).

Invasion from Lymph Nodes

The work of Adamson, 1949; Lepovetsky et al., 1953; Weiser et al., 1954; Cosnett et al., 1956; Nottingham, 1960; tend to show that microorganisms are present in lymph nodes. It is conceivable that these bacteria could constitute an internal

source to the deep tissues of post rigor meat, since upon death of the animal phagocytic activities cease and bacteria could multiply and finally spread throughout the tissues.

Nottingham (1960) observed that bacterial flora of lymph nodes and tainted meat were found to have much in common; giving further evidence for the view that bacteria are present in lymph nodes during life, and spread to surrounding tissues after death.

High correlations between rainfall and the percentage of lymph nodes containing Gram-positive rods were observed by Cosnett et al., (1956). This relationship was not observed by Nottingham (1960), however, there was a significant correlation between rainfall and the average number of bacteria in each lymph node, the bacterial load being less after a period of high rainfall than when the rainfall for the two months prior to slaughter had been low. This may be due to either a reduction in the concentration of air- and dust-borne organisms after rain (Cosnett et al., 1956), or it may be related to some nutritional factor leading to variation in resistance to infection (Nottingham, 1960). Whatever the actual mechanism by which rain reduces the bacterial load, the observed correlation gives further evidence that the bacteria found in lymph nodes gained entry into the animal before death (Nottingham, 1960).

The study of Adamson (1949) while not undertaken specifically with the subject of meat bacteriology in mind is of interest in this area. His study revealed that a large proportion of 804 lymph nodes removed from human corpses contained bacteria with coliforms, Micrococci and Streptococci predominating.

Lepovetsky et al., (1953) were able to isolate bacteria from 15 out of 23 lymph nodes, from 3 of 23 marrow samples and from 2 of 23 muscle samples from beef carcasses. The majority of the bacteria were Gram-negative rods, Gram-positive cocci, diptheroids and several types of anaerobes. Pseudomonas and Streptococcus were isolated from the muscle samples. Viable counts in the lymph nodes ranged from 70 to 764,000 per gram. The type of bacteria appeared to be of intestinal origin.

Invasion from Lungs

Few if any bacteria and fungi are resident in the trachea, bronchi and lungs (Carter, 1967). The microorganisms found in lungs of slaughtered hogs mostly appear to be the same as found in soil flora (Hulphers, 1933). Contamination of the heart blood occurs through the large veins from the lungs (Jensen, 1954).

Time of Invasion

The work of Burn (1934b) on post-mortem bacterial invasion raised the question that bacterial invasion may occur at the time of death (agonal invasion).

The work of Jensen and Hess (1941) tended to support this view that the invasion of bacteria is agonal as well as post-mortem. It was found in their study that the muscle tissue and bone marrow of living hogs are generally sterile, but that many kinds of bacteria (Bacillus, Proteus, Micrococcus, Clostridium, Achromobacter, Pseudomonas, Serratia, diptheroids and Torula) can be isolated from these sites after the animals have been slaughtered. This seemed to indicate that when the hog is stuck and bleeding out, bacteria make their appearance in blood, bone marrow and muscle.

They found as previously mentioned that the skin of swine is heavily contaminated with microorganisms and that the stick knife when passed through the skin severs the jugular vein and sometimes the carotid artery. When this occurs, the blade is washed with venous and often with arterial blood. They also found that the heart may beat from two to nine minutes after the stick wound is made. In addition, they observed that some of the hogs after being stuck contracted their heads in the direction of their forelegs, thus withholding some of the blood

by constriction and hematoma allowing some blood from this area to reach the heart and be circulated. They postulated that a negative pressure may be set up in the severed or pierced vessels, owing to the labored breathing accompanying exsanguination (oxygen starvation), the flow of the pooled blood and blood within the vein being toward the heart. They were able by dipping the blades in cultures of bacteria (possessing an identifying cultural characteristic to distinguish them from the usual flora) and then sticking the hogs in the usual manner, to recover these later from the muscular tissue and bone marrow of the carcass.

Vanderzant and Nickelson (1969) made examinations of muscle tissue of beef, pork and lamb carcasses immediately after dressing operations and after 3 days of storage. They reported that bacteria may or may not be absent from deep muscle tissues of healthy animals after slaughter. The following genera were isolated: Staphylococcus, Micrococcus, Sarcina, Streptococcus, coryneforms, Bacillus, Clostridium, Flavobacterium, Pseudomonas, Morexella, Alcaligenes, Antitratium, and yeasts and molds. They concluded from their study that contamination from exterior parts of the tissue did not play a role in contamination to the interior tissues.

PART TWO: MICROBIOLOGICAL IMPLICATIONS OF CHILLING,
STORAGE AND SHIPPING

Chilling

After the completion of the dressing operations the carcasses of meat animals normally are refrigerated immediately by suspending carcasses on overhead rails in chilling rooms.

Some plants employ the use of two chill rooms with the first room having the lowest temperatures and greater circulation of air. Since rapid cooling is necessary for extended shelf life of meat such a precooler is recommended as being beneficial for heavier beef carcasses (Patterson, 1969). The importance has also been stressed that precoolers should be of a size for a complete days kill to avoid placing warm carcasses with those already cooled (Patterson, 1969).

The procedure of rapidly chilling the carcass has not always been practiced in the meat industry as is witnessed by the comments of Moran and Smith, 1929; and Empey and Scott, 1939b; that it was the practice to allow the carcass to remain at room temperature until the animal heat had dissipated (up to 30 min.). Apparently it was thought that a carcass could be chilled too quickly, and if chilled too quickly a sort of "casing" would be formed on the outside of the carcass.

It is not generally concluded that it is not possible to chill the carcass too quickly provided no portion of it is frosted (Brandly et al., 1968).

In a comparison between the extent of the contamination acquired by beef carcasses during dressing operations and that which may be deposited on the carcass during 48 hrs. in the chill room, it has been shown that the latter frequently exceeded the former (Empey and Scott, 1939b).

Storage

The storage period is usually referred to as the period from the time the carcass has chilled until it is shipped out of the plant. As previously mentioned, storage and transportation of meat may contribute as much as 50 percent of the bacterial load on meat (Scarafoni, 1957). Patterson (1967) and Stringer et al., (1969) sampled beef carcasses at various post slaughter intervals during storage and found that the number of bacteria on the carcasses increased as time increased. Patterson (1967) reported the mean total count per gram of surface scrapings on freshly killed carcass as 3,600; after 3 days, 5,100,000; after 5 days, 130,000,000; after 6 days, 20,000,000; and after 7 days, 190,000,000. Stringer et al., (1969) reported the mean carcass count per sq. in. after 1 day storage was 17,000; after 2 days, 58,900; after 3 days 27,550; and after

4 days, 371,500. The greatest increase was on the muscle area around the aitch bone, with an appreciable increase noted in the neck region and external fat of the rounds.

Temperature

The temperature of the meat is undoubtedly the most important factor in determining the fate of microorganisms found on meat. Most of the bacteria found on meat grow well at temperatures between 15° and 40° C (59° and 104° F), with the optimum temperatures for most strains being between 35° and 40° C (95° and 104° F). At their optimum temperature the regeneration time is often as short as 20 to 30 minutes. Bacteria incubated at temperatures below their optimum will exhibit a longer generation time (American Meat Institute Foundation, 1960).

A heterogeneous population of microorganisms is present on fresh refrigerated meat (Table 1). Many of the bacteria which come in contact with meat are mesophiles. If meat is held at temperatures that permit rapid growth of these mesophilic contaminants, a heterogenic flora develops and reaches a very high total population (Ayres, 1960a).

TABLE 1

Classification of bacteria, actinomycetes, molds, and yeasts
isolated from refrigerated beef

Bacteria and Actinomyces (After Bergey's Manual of Determinative Bacteriology, 7th Ed.(9))	Molds and Yeasts
Schizomycetes	Phycomycetes
Pseudomondales	Mucorales
Pseudomonadaceae	Mucoraceae
Pseudomonas	Rhizopus
Aeromonas	Mucor
Eubacteriales	Thalassidiaceae
Achromobacteriaceae	Thamnidium
Alcaligenes	Fungi Imperfecti
Alchromobacter	Moniliales
Flavobacterium	Moniliaceae
Enterobacteriaceae	Monilia
Escherichia	Aspergillus
Aerobacter	Penicillium
Paracolonobactrum	Sporotrichum
Serratia	Dematiaceae
Proteus	Cladosporium
Salmonella	Alternaria
Micrococcaceae	Cryptococcales (after
Micrococcus	Lodder & Kreger-van
Staphylococcus	Rij (33))
Sarcina	Cryptococcaceae
Lactobacteriaceae	Torulopsis
Streptococcus	Candida
Corynebacteriaceae	Rhodotorula
Microbacterium	
Bacillaceae	
Bacillus	
Clostridium	
Actinomycetales	
Streptomycetaceae	
Streptomyces	

Source (Ayres, 1960a)

As the storage temperature is lowered, a progressively smaller proportion of the contaminating flora is able to grow. Temperatures below 7.22°C (45°F) prevent the growth of pathogenic bacteria with the exception of Clostridium botulinum type E. At storage temperatures meat develops a flora of psychrophilic bacteria that grows slowly at temperatures near freezing. At $0-2^{\circ}\text{C}$ ($32-35.6^{\circ}\text{F}$), about the only group of bacteria that grows at a significant rate are the pseudomonads, and of course they grow much slower at these temperatures. In general the bacteria that can grow at the lowest temperatures are aerobic.

Humidity

The amount of moisture loss or shrink, during chilling is held to a minimum for both economic reasons and quality retention. Therefore, it is desirable to maintain the relative humidity between 88-92% (American Meat Institute Foundation, 1960). Some reports indicate that humidity levels above this range increase the amount of slime and mold growth. Empey and Scott, (1939b) reported that surface desiccation apart from temperature reduction, may be lethal to bacteria and secondly that in the absence of surface desiccation very rapid cooling was not effective in controlling microbial growth. Ingram (1949) pointed out that the low temperature group of bacteria is

very greatly affected by humidity, tending to form a slime more readily on cut surfaces than on the skin, connective tissue or fat. Jensen (1954) stated that certain bacteria and mold growths are troublesome among cold storage meats if humidities of the coolers are not controlled. In general bacteria that can grow at the lowest temperatures usually have a high minimum of water activity.

Ayres (1956) pointed out that microorganisms appear first in damp pockets, such as the fold between the foreleg and the rest of the carcass, and their spread is greatly promoted by the condensation which occurs when a cold carcass is exposed to warm, damp air.

Stringer et al., (1969) also reported that bacteria grew faster in moister areas compared to the dryer areas of the carcass during storage.

However, there is also evidence in the literature (Scott, 1936; Ogilvy and Ayres (1951) which indicates that the influence of relative humidity has little effect in delaying microbial growth on meat surfaces. This may be explained by the fact that in a dry atmosphere the diffusion of water from the interior of the meat helps to maintain a higher moisture content near the surface than is indicated by the relative humidity of the surrounding air (Ayres, 1956).

Schmidt (1931) also observed that the surface of meat is wetter than the surrounding air, and that bacterial growth took place in low humidities calculated to increase storage life.

Frazier (1967) pointed out that if the surface of the meat is relatively dry and is allowed to exceed 10° C (50° F) Micrococcus is favored, while the pseudomonads require a high water activity for optimum growth.

Jay (1966b) lists at least eight changes that occur when muscle is converted to meat that either have or seem to have an effect upon the growth of microbes upon post mortem meats:

1. Reduction of pH by lactic acid as a result of glycolysis, thereby producing a pH less optimal for growth of most bacteria.
2. Cessation of phagocytic function making possible the growth and spread of bacteria if present in the deeper tissues.
3. Lowering the oxidation-reduction potential, making for growth of anaerobes as a result of cessation of oxygen supply. (Pseudomonas and Micrococcus are obligate aerobes and must have oxygen for growth).
4. Accumulation of various metabolites, upon the completion of rigor-mortis, which are readily utilized by many micro-organisms.
5. Combination of actin and myosin to form actomyosin which because of molecular size may be less readily attacked by bacteria than might be either component alone.
6. Autolysis or the breakdown of proteins by cathepsin creating favorable conditions for the activity of bacteria.
7. Decrease in ATP concentration, which is correlated with a decrease in water-holding capacity.

8. Release by skeletal proteins of calcium and the uptake of other metal ions such as potassium which are required by bacteria for growth.

General Cleanliness

Contamination during the chilling and storage periods apparently occurs from the same sources as during the dressing operations, (e.g., workmen, air, equipment, sawdust, floors and walls).

Empey and Scott (1939a) reported that contamination from the walls and ceilings can occur either through direct contact or by dispersion by circulating air. They found the most highly contaminated areas to be those which the sides of the carcass rubbed during filling of the room.

Floor and wall samples taken during chilling and also during the storage period by Stringer et al., (1969) showed, as would be expected, that the floors were more highly contaminated than the walls. Also, the floors and walls in the storage cooler were less contaminated than those in the pre-chilling cooler.

Fresh sawdust is used to absorb blood and fluid from the carcass as it drips to the floor. No quantitative estimate has been made of the extent of contamination derived from sawdust, but it is apparent that fine particles of this material could be carried into the circulating air, or by disturbance of the material by the feet of workers could result in the transfer of

particles to the sides of the carcasses, particularly on those areas nearest the floor.

Empey and Scott (1939a) reported that the number of organisms deposited on the carcasses from the air was invariably higher in those rooms which had sawdust on the floor. They also observed that fresh sawdust contained about 3,000,000 bacteria per gram, and of these 1 percent were considered to be psychrophilic. After exposure on the floor and increased moisture and blood the microbial populations of these samples ranged from 60,000,000 to 450,000,000 per gram.

Richardson et al., (1954) stated that during clean up in coolers where sawdust was used the air becomes permeated with mold spores which remain suspended for several hours, which could provide a source of contamination of the carcasses for the following day.

Contamination from equipment to the carcasses is acquired in a similar manner as from the equipment used in slaughtering and dressing. (Voegeli et al., 1953 and Stringer et al., 1969).

Genera of Microorganisms Found on Chilled Meat

Apparently Tissier and Martelly (1902) were the first to make a comprehensive study of the flora of meat. As previously stated many of the early studies led to faulty conclusions due to defective bacteriological methods.

Other early investigators (Haines, 1933a, b; Empey et al., 1934, 1939a, b; Jepsen, 1947) reported that a large percentage of the bacteria found on refrigerated fresh meat were of the genus Achromobacter. In 1948, the classification of Pseudomonas and Achromobacter was changed, and since that time most researchers have reported Pseudomonas to be the principle genus found on fresh meat (Ayres, 1951, 1955, 1960a; Kirsch et al., 1952; Jensen, 1954; Wolin et al., 1957; Brown et al., 1958; Halleck et al., 1958c; Stringer et al., 1966, 1969).

Ayres et al., (1950) were the first to suggest that due to changes in classification made in the 6th Bergey's Manual (1948), a number of types of organisms which were previously reported as being Achromobacter, no doubt would be classified as members of the genus Pseudomonas in the present scheme.

To substantiate this theory, Brown and Weideman (1958) made a reassessment of 129 cultures derived from the original isolations of Empey and Scott (1939a), which were kept in the laboratory for two decades. They also isolated 60 additional cultures from beef held at low temperatures. Of all bacteria studied 93 percent were found to be species of Pseudomonas.

The results of this work corrected the earlier statement of Empey and Scott (1939a) that the genus Achromobacter accounted for 90 percent of the psychrophilic bacteria on Australian beef.

These results also supported the findings, of the later workers previously mentioned, that Pseudomonas is the principle organism growing on refrigerated beef at low temperatures.

Organisms belonging to many different genera occurring as contaminants of refrigerated meat have been identified (Table 1) by Ayres (1960a).

It was found that only Pseudomonas, Achromobacter, Flavobacterium, Micrococcus, Microbacterium, and Penicillium were regularly found from examinations made from a large number of isolates from refrigerated beef, Pseudomonas and Micrococcus being predominant during the storage period (Ayres, 1960a).

As previously mentioned there is a relation between the numbers and type of flora developing on meat during storage and the temperature of storage (Ayres, 1959; Stringer et al., 1969). Pseudomonas predominated on meat kept at 0° C, 5° C, and 10° C (32°, 41°, and 50° F) while at 15° C (57.2° F) there was approximately an equal incidence of Pseudomonas and Micrococcus (Ayres, 1960a).

Occasionally there were representatives of the genera Achromobacter, Flavobacterium, Bacillus, Clostridium, and Streptococcus, but the enterobacteria and staphylococci were not recovered from meat after several days of storage at temperatures below 10° C (50° F).

Penicillium were more commonly isolated from beef than were any other species of molds. Cladosporium, Thamnidium, Mucor and Rhizopus were next in order. Aspergillus was encountered occasionally on meat held at temperatures of 10° C (50° F) or higher. Very rarely isolations of Monilia, Alternaria and Sporotrichium were made. For the most part the activity of molds is restricted to the outer surfaces where aerobic conditions are maintained. After several days of refrigeration, Penicillium and Cladosporium often were found growing on the connective tissue or on the native layer of fat covering the muscle tissue. Both organisms can produce spots ranging in color from yellow to black although discolorations attributed to Cladosporium ordinarily are darker in color than those identified as Penicillium.

The most encountered species of Pseudomonas is Pseudomonas geniculata, P. fragi and P. rugosa (Kirsch et al., 1952; Wolin et al., 1957; Ayres 1960a; and Stringer et al., 1969).

Strains of organisms belonging to three genera of false yeasts -- Torulopsis, Candida, and Rhodotorula -- were found occasionally on refrigerated meat; no true yeasts were found (Ayres, 1960a).

Microbial Levels and Spoilage

Various workers have referred to certain minimum concentrations of bacteria at the time that incipient spoilage becomes apparent (Schmidt, 1931; Empey and Vickery, 1933; Haines, 1933b; Moran, 1935; Kraft and Ayres, 1952). Counts ranged from 10,000,000 to 100,000,000 per sq. cm. of surface. Haines (1933b) and Empey and Vickery (1933) considered that slime point was attained when the surface loads were 32,000,000 and 50,000,000 per sq. cm. respectively. Schmidt (1931) reported ranges from 50,000,000 to 100,000,000 per sq. cm. Kraft and Ayres (1952) reported that the first off-odor could be detected when the surface count approximated 2,000,000 per sq. cm. and definite off-odor could be recognized when surface counts of 10,000,000 per sq. cm. were reached.

Ayres (1951) reported that during the first day or two of storage at 4.4°C (39.9°F) the bacterial counts showed an initial decline before microorganisms began to proliferate. Presumably, this temperature may be unstable for survival or growth of organisms other than the spoilage types and, in the early hours of storage, insufficient time has elapsed for the psychrophilic organisms to replace losses of the mesophilic organisms.

Moran and Smith (1929) reported a negligible increase in numbers of organisms in the deep tissues of beef stored for 2 weeks at 5° C (41° F) which led them to conclude that spoilage by bacteria in the deeper parts of the flesh is unimportant compared with that at the surface.

Haines (1933b) offered a possible explanation for the growth on uncut surfaces being limited, namely that the surface of the whole or quartered carcass is covered by a layer of fat having poor nutrient properties.

Storage time has been shown to be lengthened by storage of meats in an atmosphere containing carbon dioxide or ozone. Although considerable experimental work has been done on the gas storage of fresh meats (Tomkins, 1932; Coyne, 1933;) the method has not been used extensively. There is no agreement upon the optimal concentration of carbon dioxide to be effective. Recommendations vary from 10 to 30 percent for most meat. Increased amounts of carbon dioxide in the atmosphere increasingly inhibits microorganisms but also hastens the formation of metmyoglobin and methemoglobin and hence the loss of "bloom" or natural color.

Storage time has been increased by using 2.5 to 3 ppm of ozone in the atmosphere. At these levels microorganisms will be inhibited, but higher concentrations are needed to stop

growth already begun (Frazier, 1967). A disadvantage of ozone is that it is an oxidizing agent, and will give rise to flavors in fats.

Since ozone masks odor, thereby interfering with inspection, equipment that produces ozone in appreciable amounts may be used only in coolers set aside solely for aging of meat. The concentration of ozone in the air should not, at any time, exceed 0.1 ppm (Manual of Meat Inspection Procedures, United States Department of Agriculture, 1968).

In a study of microbial changes in meat during aging at elevated temperatures, Rey et al., (1970) observed that Staphylococci were recovered from carcasses more frequently that were aged at elevated temperatures (60.8° and 71.6° F). Conventional low temperature treatment yielded retail cuts with better keeping quality than did the higher temperatures of aging.

Most of the pathogens are mesophilic and can grow well at elevated temperatures, even for psychrophiles the rate of multiplication has been reported to increase with increasing temperature within a certain range (Ingraham, 1958; Straka et al., 1960).

Low incidences (0.8 percent) of Clostridium perfringens have been reported on raw meat (Hall and Angelotti, 1965). There are also indications that this organism does not multiply

in the temperature range of 5-15° C (41°-59° F) (Hall and Angelotti, 1965; Rey et al., 1970).

Greenberg et al., (1966b) reported a very low incidence (.042 percent of 2,358 samples) with Clostridium botulinum in raw meat at packing house levels on the North American continent.

Shipping

The same factors affecting bacterial multiplication apply during transportation. Stringer et al., (1969) reported a significant increase in microbial numbers during transportation from the packing plant to the retail store (from mean counts per sq. in. of 60,300 before shipment to 871,000 upon arrival at the retail store). This was attributed to contamination through handling and changes in meat temperature during transportation. Season also had an effect on the degree of contamination upon receipt at the retail store with mean counts per sq. in. of 2,240,000 in winter as compared to 398,000 in summer.

PART THREE: MICROBIOLOGICAL IMPLICATIONS OF CUTTING, COMMINUTING, PACKAGING AND FREEZING FRESH MEAT

Further operations such as cutting, comminuting, packaging and freezing must be hygienically carried out. Through this additional handling the microbial level of meat increases.

Cutting

Ayres (1956) indicated three important differences which serve to increase the bacterial loads in the cutting room:

(1) cut surfaces and juices support growth of large bacterial populations; (2) the microorganisms which have become entrenched and have multiplied during evisceration and the chill room are redistributed by cutting, and (3) much larger amounts of surface are exposed to potential contamination.

As with other areas previously mentioned, bacteria in the air in the cutting room may play a significant part in contributing contamination to the meat as well as to the equipment. By exposing Petri dishes for 10 minute intervals at table top heights in the cutting rooms in retail stores at the beginning, middle and end of each day's operation, Stringer et al., (1969) found the air nearly free of contamination at the beginning of each day. Air contamination increased over 3-fold (from a mean count of 8 to 28) as the working day progressed. By mid-day the air contamination was at its maximum.

Apparently equipment concerned directly with the cutting operations (knives, saws, boning blocks, slicers, table tops, etc.) offered the greatest source of contamination for fresh meat during the cutting operations (Voegeli et al., 1953; Stringer et al., 1969). The bacterial level of those surfaces

not directly involved in the operation such as walls were relatively low (Voegeli et al., 1953).

Stringer et al., (1969) observed that considerable contamination was transferred to the meat during the cutting operations especially when the saw blade became soiled by cutting of the meat product.

No literature could be found in regard to the contribution to bacterial levels that boning operations or personnel engaged in these operations made.

The wholesale and retail cuts of fresh meat have a mixed flora. The most common are members of the genus Pseudomonas or a closely related genera Achromobacter. Other genera of bacteria that are frequently encountered are Lactobacillus, Microbacterium, and Micrococcus.

If meat is packed in such a way that much of the surface is not exposed to air, Lactobacillus and Microbacterium organisms form a greater portion.

Comminuting

Ground or comminuted meats such as fresh ground beef and fresh pork sausage provide a highly favorable environment for the multiplication of bacteria. Results of the many surveys of ground meat for sale in retail markets justify the prevalent idea that ground meat is often of dubious bacteriological quality.

From the work done in this and other countries especially Britain (Dyett and Shelly, 1962; Board et al., 1966; and Dowdell and Board, 1967, 1968) it appears that such products share a common feature -- that of large microbial populations.

In an attempt to account for the large number of bacteria so generally found in such products, LeFevre (1917) listed four factors to be considered:

- (1) The kind of meat used in these type products. Many dealers utilize such products as a profitable method of disposing of the cheapest grades or otherwise unsalable meat.
- (2) The increased susceptibility offered by ground meats to the growth and multiplication of microorganisms. Fragmentation of tissues with the liberation of cell contents and the intimate mixing of the bacteria normally found on the meat surface with the macerated tissues results in a product subject to rapid modification by bacteria.
- (3) The lack of cleanliness in the preparation and handling of the meat. Dirty grinders, knives, paddles, and hands of personnel, all contribute their share towards the bacterial content of the finished product.
- (4) Exposure to contamination after the product is prepared and waiting for sale.

Another factor mentioned by Rogers and McClesky (1957) is that time and temperature of storage also have a very important bearing on bacterial populations.

The obvious relationship of bacterial numbers to quality led to several investigations as to bacterial content of ground meats during the early part of the 20th Century.

Most of this early work was concerned with the numbers and some of the authors suggested sanitary standards bases on bacterial counts (Marxer, 1903; Weinzirl and Newton, 1914a,b; Carey, 1916; LeFevre, 1917; Elford, 1936; and Tobey, 1944). These standards have not received wide acceptance, however.

Marxer (1903) suggested that a limit of 1,000,000 bacteria per gram be set. Weinzirl and Newton (1914a,b) found this standard too low and suggested that a maximum of 10,000,000 organisms per gram determined at 20° C (68° F) be allowed in ground beef. They concluded, however, that there was no close agreement between the actual number of bacteria and degree of spoilage. Carey (1916) concluded that the determination of mere numbers of bacteria in meat had little significance because numbers per gram of sausages varied so widely. LeFevre (1917) suggested that the bacterial count could be of value in detecting the use of improper materials and defects in handling of the product. He concluded that 1,000,000 bacteria per gram determined at 25° C (77° F) was a reasonable maximum allowed for ground beef.

Hoffstadt (1924) reported no correlation between counts and organoleptic factors, and concluded that bacterial counts as a standard for meat analysis could be eliminated for comminuted fresh meat products.

Elford (1936) suggested a maximum allowable count of 10,000,000 per gram determined at 37° C (98.6° F). Tobey (1944) stated that good hamburger should have a total count not exceeding 800,000 per gram and not more than 200 coliforms per gram.

Kirsch et al., (1952) made a study to see if modern emphasis on food inspection, sanitation and wide spread use of refrigeration has made a difference in quality of ground beef since the work of these before mentioned early workers. They found it to be little if any better than that of the product available many years ago. From their investigations of 20 samples of market hamburger they obtained aerobic plate counts which ranged from 1,400,000 to 95,000,000 organisms per gram.

Work in more recent years has considered kinds as well as numbers of microorganisms in fresh ground meat at the time of manufacture (Sulzbacher and McLean, 1951); during storage (Sulzbacher and McLean, 1951; Miller, 1955; Rogers and McClesky, 1957); at the time of purchase (Greer, 1932; Foltz, 1941; Kirsch et al., 1952; Rogers and McClesky, 1957); and at the time of spoilage (Hoffstadt, 1924; Stuart et al., 1945; Kirsch et al., 1952).

Microflora at Time of Manufacture

Sulzbacher and McLean (1951) studied the taxonomic distribution of the bacteria found in 316 samples of commercially produced fresh pork sausage at the time of manufacture (Table 2). More than 74 percent of the isolates belonged in six genera. These are shown in Table 3, together with a key to their relative prevalence in either freshly prepared sausage, older samples stored at home refrigerator temperatures of 5-8° C (41-46.4° F), spices or plant equipment. All 316 samples were tested for the presence of Salmonella with negative results.

Greer (1932) reported counts on samples of freshly ground beef to range from 8,500,000 to 24,300,000 per gram.

Board et al., (1966) also pointed out that unsatisfactory handling of ground product can be expected to favor growth of the Pseudomonas-Achromobacter types. They also noted that the incidence of these organisms tended to increase with an increase in overall contamination at time of manufacture.

Microflora While in Storage

Sulzbacher (1950) reported that certain mesophilic organisms (Salmonella anatum, Salmonella choleraesuis, Escherichia coli, and Micrococcus pyogenes) did not grow in either fresh or frozen and thawed ground pork held at 44.6° F (7° C) for a period of 5 days.

TABLE 2

Generic Distribution of Organisms Isolated from Pork Sausage

Genus	Number of Isolates	Percent of Total
<i>Pseudomonas</i>	34	10.8
<i>Xanthomonas</i>	4	1.1
<i>Micrococcus</i>	3	1.0
<i>Sarcina</i>	5	1.6
<i>Neisseria</i>	1	0.3
<i>Microbacterium</i>	47	14.9
<i>Alcaligenes</i>	19	6.0
<i>Achromobacter</i>	40	12.7
<i>Flavobacterium</i>	8	2.5
<i>Escherichia</i>	2	0.6
<i>Paracolobactrum</i>	2	0.6
<i>Aerobacter</i>	2	0.6
<i>Proteus</i>	21	6.7
<i>Bacterium</i>	65	20.6
<i>Bacillus</i>	28	8.9
Misc. unidentified	28	8.9
Yeasts	<u>7</u>	<u>2.2</u>
Total	316	100.0

Source Sulzbacher and McLean, (1951)

TABLE 3

Habitat Distribution of Bacteria Isolated

Genus	Number of Isolates	Percent of Total	New Sausage	Old Sausage	Spices	Equipment
Pseudomonas	34	10.8	+++	---	---	+
Microbacterium	47	14.9	++	++++	---	---
Alcaligenes	19	6.0	+	++	---	---
Achromobacter	40	12.9	++	++	---	+
Bacterium	65	20.6	+++	++	---	+
Bacillus	28	8.9	+	+	++	+
Total	233	74.1				

Source Sulzbacher and McLean, (1951)

Sulzbacher and McLean (1951) were unable to recover significant numbers of Pseudomonas from fresh pork sausage samples stored at 5-8° C (41-46.4° F). They reported that Microbacterium made up a rather large proportion of the flora during storage at these temperatures (Table 3). Alcaligenes were slightly more prevalent in the stored samples than in freshly prepared product. They postulated that due to their prevalence and persistence during storage that members of the genus Microbacterium may be responsible for the development of the acid taste in stored sausage. Aerobic plate counts ranging from 1,400,000 to 95,000,000 per gram were reported.

Rogers and McClesky (1957) observed that members of the genera Pseudomonas and/or Achromobacter were most abundant in ground beef after 14 days storage at 7° C (44.6° F) (Table 4). Similar results have been reported by Kirsch et al., (1952). Organisms that grew most abundantly during storage at 0° C and 2° C (32° and 35.6° F) were of the genus Pseudomonas.

Halleck et al., (1958a) reported that fresh ground pork with an initial count of 65,000 per gram, then packed in cans and stored at 1-3.5° C (33.8-38.3° F) had a bacterial count of 1.4 million per gram after 14 days. At that time Pseudomonas-Achromobacter types out numbered the lactobacilli 7 to 1. In a similar experiment, ground pork with an initial count of 130 per

TABLE 4
Changes in bacterial flora of ground beef
stored at 7° C (44.6° F)

ORGANISM	Isolated at 37° C		Isolated at 7° C	
	0 days	14 days	0 days	14 days
Pseudomonas and, or Achromobacter	11	64	4	84
Bacillus	8	8	28	6
Flavobacterium	0	6	0	0
Microbacterium	0	7	0	5
Micrococcus	50	14	26	5
Sarcina	6	1	0	0
Yeasts	25	0	42	0
Totals	100	100	100	100

Source Rogers and McCleskey, (1957)

gram was stored 14 days at 4.5 to 6.5° C (40.1-43.7° F). The count after this holding period was 19 million per gram, mainly Pseudomonas-Achromobacter types.

Dyett and Shelly (1962) reported that British sausage wrapped in cellulose film yielded 1,000,000 organisms per gram in 3-4 days storage at 22° C (71.6° F) when a sulphite preservative was added. Approximately the same count was obtained without a preservative stored 3 to 5 days at 3-5° C (37.4-41° F).

Microflora at time of Purchase

Greer, (1932) reported counts in unfrozen ground beef at the time of purchase to range from 6,000,000 to 43,700,000 bacteria per gram.

Rogers and McClesky (1957) in a study of bacteriological quality of ground beef in retail markets found in 96 samples of ground beef purchased in local markets an average of 32,800,000 bacteria per gram when counted at 37° C (98.6° F) after 2 days incubation and 192,500,000 per gram when incubated at 7° C (44.6° F) for 7 days. The highest count at 37° C (98.6° F) was 230,000,000 and the lowest was 1,600,000 per gram. Samples obtained from larger markets usually contained fewer bacteria than those from smaller ones. Micrococcus, Bacillus, and yeasts were the most prevalent organisms.

Miller (1964) in a study of 10 different brands of self-service, prepackaged, shipped-in fresh pork sausage (227 samples) found that counts ranged from 10,000 to 180,000,000 per gram. Microbacteria and lactobacilli dominated the flora in the majority of packages of wrapped-roll sausages. Conversely, Pseudomonas-Achromobacter types dominated in most of the skinless link-type sausages.

Dowdell and Board (1968) in a survey of British fresh sausages (both diced beef and pork) at the retail level found a similarity between pork and beef sausages as to numbers. Counts ranged from 100,000 to 500,000,000 per gram, with Microbacterium thermosphactum predominant in the majority of the samples. In samples containing greater than 100,000,000 organisms per gram, members of the Pseudomonas-Achromobacter complex tended to be plentiful.

The absence of Flavobacterium was surprising because these organisms can grow near 0° C (32° F) and have been reported in studies on microflora during dressing and storage (Empey and Scott, 1939a; Ayres, 1960a).

Kirsch et al., (1952) reported counts at the time of the first noticeable sour odor to be 500,000,000 per gram.

Coliforms as Indicators in Comminuted Meats

As with so many foods the number of coliforms in comminuted fresh meat has been accepted as a general index of hygiene during its preparation. The value of such categories might be questioned especially in view of the results found by the various workers, as the numbers varied widely and did not correlate well with the total counts.

Foltz (1941) in sampling ground pork and beef purchases from retail markets reported that 10 percent of the samples tested were free of coliform bacteria and of the remaining samples 9 percent contained E. coli alone; and 40 percent contained a mixture of E. coli and Aerobacter aerogenes.

Miller (1955) found 1,500,000 Gram-negative psychrophilic bacteria per gram in unseasoned ground pork prepared from a carcass stored 10 days at 1° C (33.8° F). Numbers decreased to 40,000 per gram in 10 months at -17.8 to -22° C (0° to -7.6° F).

Rogers and McClesky (1957) found coliforms in all samples (96 from 24 retail markets). Numbers varied from 20 to 1,000,000 per gram, the average was 52,000 per gram.

Dyett and Shelley (1962) found high numbers of coliforms in British fresh sausage but in contrast to what Dowdell and Board (1968) reported, they found that coliforms multiplied readily during storage of sausage.

Dowdell and Board (1968) reported that coliform counts ranged from 130 to 240,000 per gram. They also reported that a similarity was found between pork and beef sausages as to numbers of coliforms present. They concluded that coliforms appear to be common contaminants of such ground meat products.

Dowdell and Board (1968) reported that their numbers do not change appreciably during storage, and suggested that the behavior of coliforms may be influenced by competitive pressures of the dominant contaminants.

Packaging

Halleck et al., (1958a) investigated the bacterial flora of prepackaged meats. They used a number of packaging materials and stated that the packaging material did not selectively affect the rates of growth of the predominant genera. Two groups of bacteria dominated the bacterial flora of the fresh meat during a four-week storage at 34-38° F (1.11-3.33° C). They reported that during the first two weeks Lactobacillus and non-pigmented organisms of the Pseudomonas-Achromobacter type predominated, whereas in the latter part of the storage period those of the Pseudomonas fluorescens type became predominant.

In a later publication Halleck et al., (1958b) reported further on their work with additional films. This time they reported that the packaging materials had an effect on bacterial

growth. The water and gas impermeable packaging films permitted rapid growth until dehydration and mold interferred. The essentially impermeable packaging films increased the lag phase, and the final bacterial counts were not as high as with the more permeable films. In the preliminary phase of this study it was found that the ground beef packaged in a gas-impermeable wrapper tended to sour after extended storage (15-18 days). This was attributed to the growth of lactic acid type bacteria.

Lactobacilli may occur in fresh prepackaged meat such as lamb, beef and pork (Halleck et al., 1958c). Vacuum packaging of meat products in oxygen impermeable films is a favorable factor in encouraging the growth of lactobacilli. The anaerobic conditions favor the growth of these organisms at the expense of aerobic species, although Ingram (1962) pointed out that aerobic micrococci can obtain their oxygen from reduction of nitrate to nitrite instead of from the air.

Jay et al., (1962) also found that ground beef retained acceptability longer in an essentially air-impermeable film such as Saran than in cellophane.

Baran et al., (1970) found that vacuum packaging of hamburger resulted in bacterial growth attaining a stationary phase after six days of storage, whereas hamburger packaged in air-permeable films continued development of aerobes.

Vacuum-packaging appeared to have little effect on aerobic counts in fresh hamburger. This may have resulted because hamburger has air incorporated in the meat. They also found that anaerobic bacteria were able to grow in meat packaged with all types of films used regardless of oxygen permeability.

Freezing

The original reason for freezing meat was that spoilage due to the growth of microorganisms both in and on the meat was prevented, thereby increasing its storage life from weeks to months. It must be recognized that freezing does not necessarily kill microorganisms and that they can grow slowly at temperatures well below 0° (32° F) provided that a source of food is available. Thus, on supercooled media, bacteria have been grown at -5° C (23° F) and even at -7° C (19.4° F) (Haines 1930, 1931a,b, 1932, 1937). Bacterial growth can occur on frozen meat down to -3° C (26.6° F) and yeasts and molds can grow at temperatures as low as -7° C (19.4° F) (Callow, 1952).

At temperatures below -7° C (19.4° F) no growth of microorganisms has been observed and it is for this reason that -10° C (14° F) is accepted by the meat industry as a safe temperature for the cold storage of frozen meat (Callow, 1952).

Many forms of microorganisms have been found growing on frozen meat when the conditions of storage have been faulty

(Wright, 1912, 1923; Brooks and Kidd, 1921) and in any case, the surface of the meat will be contaminated by microorganisms before freezing. Freezing itself may kill some of these and others may die during storage (Haines, 1938). There is evidence that denaturation of proteins plays a part in killing bacteria during cold storage below -7°C (19.4°F) and that as consequence higher temperatures of storage give more rapid rates of killing than lower ones (Callow, 1952). Frozen meat when defrosted after storage is cleaner microbiologically than when it was frozen but is still not sterile (Callow, 1952). Jensen (1954) stated that when frozen meats were held at 24°F (4.44°C) there was a greater destruction of bacterial cells, than at -22°F (7.6°C), especially after 3 weeks storage.

A very widespread popular belief holds that the rate of microbial growth is much faster after freezing and thawing than the unfrozen. Consumers are frequently cautioned to use frozen foods promptly after thawing. These warnings often imply that there may be a serious public health hazard in holding thawed frozen meat for any length of time and that refreezing thawed frozen meat is particularly dangerous. With this in mind Sulzbacher (1953) found that the growth rate of psychrophilic organisms in ground meat that had been frozen and thawed was in fact slower than in unfrozen ground meat. They also found that

Salmonella, Micrococcus and Escherichia did not grow in either fresh or frozen and thawed ground pork held at 7° C (44.6° F) for a period of 5 days. These results would seem to indicate that there is no scientific basis for the commonly held belief that frozen meat becomes more perishable after thawing than fresh meat.

CONCLUSION

One of the values derived from a review is that in evaluating the work that has been accomplished, attention is called to a much greater task which still remains to be done. More study is needed to ascertain the significance and fate of pathogens that are acquired by the meat. There is still too little published work dealing with bacterial contamination on which to base standards. There is obviously no easy way of producing meat carcasses with low numbers of microorganisms present on the meat. To maintain complete sterility appears to be hopeless, but to maintain the contaminants at a minimum offers enticing possibilities. More work needs to be done toward ways of preventing contamination. However, any new techniques must be compatible with the necessity for rapid and economical handling of the meat.

REFERENCES

- Adamson, C. A. 1949. A bacteriological study of lymph nodes. *Acta Med. Scand.* 227: 1-273.
- Ahern, D. G., and W. Kaplan. 1969. Occurrence of Sporotrichum schenckii on a cold-stored meat product. *Amer. J. Epidem.* 89: 116-124.
- Akers, J. M. 1949. Developments in slaughterhouse practice. *Food Manufacture.* 44:1, 32.
- Alford, J. A. and S. A. Palumbo. 1969. Interaction of salt, pH, and temperature on the growth and survival of Salmonella in ground pork. *Appl. Microbiol.* 17: 528-532.
- American Meat Institute Foundation. 1960. The Science of Meat and Meat Products. p. 151-179. W. H. Freeman and Company, San Francisco.
- Anderson, E. S., N. S. Galbraith, and C. E. D. Taylor. 1961. An outbreak of human infection due to Salmonella typhimurium phage type 20a associated with infection in calves. *Lancet* 22: 854-855.
- Arnold, L. 1928. The passage of living bacteria through the wall of the intestine and the influence of diet and climate upon intestinal auto-infection. *Am. J. Hyg.* 8: 604-632.
- Ayres, J. C., W. S. Ogilvy, and G. F. Stewart. 1950. Post-mortem changes in stored meats. I. Microorganisms associated with development of slime on eviscerated cut-up poultry. *Food Technol.* 4: 199.
- Ayres, J. C. 1951. Some bacteriological aspects of spoilage of self-service meats. *Iowa State College J. Sci.* 26: 31.
- Ayres, J. C. 1955. Microbiological implications in the handling, slaughtering, and dressing of meat animals. *Advances in Food Res.* 6: 109-161.
- Ayres, J. C. 1956. The source and significance of microorganisms on meats. *Proc. Ninth Annual Reciprocal Meat Conference.* No. 9, p. 165.

- Ayres, J. C. 1959. Use of coating materials or film impregnated with chlortetracycline to enhance color and storage life of fresh beef. *Food Technol.* 13: 512.
- Ayres, J. C. 1960a. Temperature relationships and some other characteristics of the microbial flora developing on refrigerated beef. *Food Res.* 25: 1.
- Ayres, J. C. 1960b. The relationship of organisms of the genus Pseudomonas to the spoilage of meat, poultry and eggs. *J. Appl. Bacteriology.* 23: 471-486.
- Ayres, J. C. 1967. Use of fluorescent antibody for the rapid detection of enteric organisms in egg, poultry and meat products. *Food Technol.* 21: 631-635.
- Badaway, A. M., R. M. Campbell, D. P. Cuthbertson, and B. F. Fell. 1957. Changes in the intestinal mucosa of the sheep following death by humane killer. *Nature.* 180: 756.
- Baran, W. L., A. A. Kraft, and H. W. Walker. 1970. Effects of carbon dioxide and vacuum packaging on color and bacterial count of meat. *J. of Milk and Food Technol.* 33: 3, 77-82.
- Barail, L. C. 1947. Bacteriology of food packaging materials. *Modern Packaging.* 21: 145.
- Barlow, J. and A. G. Kitchell. 1966. A note on the spoilage of prepackaged lamb chops by Microbacterium thermosphactum. *J. Appl. Bact.* 29: 185.
- Barnes, E. M. and M. Ingram. 1955. Changes in the oxidation-reduction potential of the sterno-cephalicus muscle of the horse after death in relation to the development of bacteria. *J. Sci. Food Agri.* 6: 448.
- Barraud, C., A. G. Kitchell, H. Labots, G. Reuter, and B. Simonsen. 1967. Standardization of the total aerobic count in meat and meat products. *Fleischwirtschaft.* 47:1313.
- Berry, J. 1956. Bacteriology of frozen foods. *J. Bact.* 51: 639.
- Blackwell, F. O. 1969. The biggest problem in food hygiene-people. *J. of Milk and Food Technol.* 32: 6, 172-175.

- Blom, T. 1957. Methods of stunning, slaughter, and collection of blood. In: Meat Hygiene. WHO Monograph Series No. 33, Geneva, p. 137-145.
- Board, R. G., E. L. Egar, L. Y. Young, and E. G. H. Wilson. 1966. A Microbiological survey of British fresh sausages. J. Appl. Bacteriol. 29: 3, ii.
- Borton, R. J., N. B. Webb, and L. J. Bratzler. 1968. The effect of microorganisms on the emulsifying capacity and extract release volume of fresh porcine tissues. Food Technol. 22: 94-96.
- Bothast, R. J., H. W. Ockerman and V. R. Cahill. 1968. Improved procedures for meat sanitation. J. Milk and Food Technol. 31: 340-343.
- Brandly, P. J., G. Migaki, and K. E. Taylor. 1968. Meat Hygiene, 3rd Ed. Lea and Febiger, Philadelphia.
- Brewer, C. M. 1925. Bacteriological content of market meat. J. Bact. 10: 543-549.
- Brooks, F. T. and M. N. Kidd. 1921. The "black spot" of chilled and frozen meat. Dept. Sci. Ind. Res. Food Invest. Board (Brit.) Special Rept. No. 6.
- Brooks, F. T. and B. A. Hansford. 1923. Mould growth upon cold-storage meat. Dept. Sci. Ind. Research Food Invest. Board (Brit.) Special Rept. No. 17.
- Brown, A. D. and J. F. Weidemann. 1958. The taxonomy of the psychrophilic meat-spoilage bacteria: a reassessment. J. Appl. Bacteriol. 21: 11.
- Brune, H. E., 1970. Salmonella survey of a beef slaughtering operation. (unpublished).
- Brune, H. E., and L. S. Oldham. 1970. Swine slaughtering operations equipment contamination survey. (unpublished).
- Bullerman, L. B. and J. C. Ayres. 1968. Aflatoxin producing potential of fungi isolated from cured and aged meats. Appl. Microbiol. 16: 1945.

- Bullerman, L. B., P. A. Hartman, and J. C. Ayres. 1969a. Aflatoxin production in meats: stored meats. *Appl. Microbiol.* 18: 714-717.
- Bullerman, L. B., P. A. Hartman and J. C. Ayres. 1969b. Effect of temperature on aflatoxin production in meats. *Appl. Microbiol.* 18: 717-722.
- Bulloch, W. 1938. *The History of Bacteriology*. London.
- Burke, M. V., K. H. Steinkraus, and J. C. Ayres. 1950. Methods for determining the incidence of putrefactive anaerobic spores in meat products. *Food Technol.* 4: 21-24.
- Burn, C. G. 1934a. Post-mortem bacteriology. *J. Infect. Dis.* 54: 388.
- Burn, C. G. 1934b. Post-mortem bacterial invasion. *J. Infect. Dis.* 54: 395.
- Burn, C. G., and L. W. Burket. 1938. Comparative bacteriologic studies of human blood, viscera and teeth obtained at necropsies. *Arch. Path.* 25: 643-651.
- Butler, O. D., L. J. Bratzler, and W. L. Mallmann. 1953. Effect of bacteria on the color of prepackaged retail beef cuts. *Food Technol.* 7: 397-400.
- Callow, E. H. 1952. Frozen meat. *J. of Sci. Food Agri.* 3: 4, 145-150.
- Callow, E. H., and M. Ingram. 1955. *Food*. 24: 52. (Cited by Nottingham, 1960).
- Carey, W. E. 1916. The bacterial examination of sausages and its sanitary significance. *Am. J. Pub. Health.* 6: 124-135.
- Carter, G. R. 1967. *Veterinary Bacteriology and Mycology*. Thomas, Springfield, Illinois.
- Casman, E. P., D. W. McCoy, and P. J. Brandly. 1963. Staphylococcal growth and enterotoxin production in meat. *Appl. Microbiol.* 11: 498-500.

- Cherry, W. B., M. Scherago, and R. H. Weaver. 1943. The occurrence of Salmonella in retail meat products. Amer. J. Hyg. 37: 210-211.
- Childers, A. B. and E. E. Keahey. 1970. Sources of Salmonella contamination of meat following approved livestock slaughtering procedures. J. of Milk and Food Technol. 33: 1, 10-12.
- Cockrill, W. R. 1963. International problems and practices in meat hygiene. Vet. Rec. 75: 875-883.
- Coleby, B., M. Ingram, D. N. Rhodes, and H. J. Shepherd. 1962. Treatment of meats with ionizing radiations: I. The irradiation preservation of pork sausages. J. Sci. Food Agri. 13: 628-633.
- Cosnett, L. S., D. H. Hogan, N. H. Law, and B. B. Marsh. 1956. Bone-taint in beef. J. Sci. Food Agric. 7: 546-551.
- Costilow, R. N., B. A. Batshon, L. J. Bratzler, and D. A. Robach. 1955. Interactions between ascorbic acid and psychrophilic bacteria associated with the discoloration of prepackaged beef. Food Techn. 9: 560-563.
- Coyne, F. P. 1933. The effect of carbon dioxide on bacterial growth. Proc. Roy. Soc. (London). Ser. B, 113: 196.
- Deatherage, F. E. 1957. Use of antibiotics in the preservation of meats and other food products. Amer. J. Public Health. 47: 594.
- DeBeukelaer, F. L. and H. Wang. 1955. Dehairing of hogs. American Meat Institute Foundation Circular No. 32.
- Deibel, R. H., N. J. Jacobs, J. Yao, and C. F. Niven Jr. 1963. Bacteriology of cervical lymph node abscesses in swine. Proc. 15th. Res. Conf. Am. Meat Inst. Foundation, Univ. of Chicago. p. 17-22.
- Deibel, R. H. 1964. The group D. streptococci. Bacteriol. Rev. 28: 330.
- Dolman, C. E. 1957. The Epidemiology of meat-borne disease. In: Meat Hygiene. WHO Monograph Series No. 33, p. 11-108.

- Dowdell, M. J., and R. G. Board. 1967. Changes in microflora during storage of sausages. *J. Appl. Bacteriol.* 30: Suppl. 2-3.
- Dowdell, M. J., and R. G. Board. 1968. A microbiological survey of British fresh sausage. *J. Appl. Bacteriol.* 31: 378-396.
- Dubois, C. W. and D. K. Tressler. 1940. Influence of rates of freezing and temperature of storage on quality of frozen meat. *Institute of Food Technol. Proc. 1st Conf.* p. 167.
- Dunker, C. F. and O. G. Hankins. 1953. Rates of freezing and thawing meats. *Food Technol.* 7: 505.
- Dyett, E. J. and D. Shelly. 1962. Microbiology of British fresh sausage. *J. of Sci. Food Agri.* 13: 166. (Abst).
- Dyett, E. J. 1963. Microbiology of raw materials for the meat industry. *Chem. Ind.* 6: 234-237.
- Elford, W. C. 1936. Bacterial limitations in ground fresh meat. *Am. J. Pub. Health.* 26: 1204-1206.
- Elliott, R. P. 1970. Microbiological criteria in U.S.D.A. regulatory programs for meat and poultry. *J. of Milk and Food Technol.* 33: 5, 173-177.
- Ellis, E. M. 1962. Salmonellosis in Florida cattle. *Proc. U. S. Livestock Sanit. Assn. 65th Annual Meeting, Minneapolis.* p. 161-163.
- Empey, W. A. and J. R. Vickery. 1933. The use of carbon dioxide in the storage of chilled beef. *J. Australian Council Sci. Ind. Research.* 6: 233-243.
- Empey, W. A., W. J. Scott, and J. R. Vickery. 1934. The export of chilled beef. The preparation of the "idomenous" shipment at the Brisbane abattoir. *Aus. J. Council Sci. Ind. Research.* 7: 73.
- Empey, W. A. and W. J. Scott. 1939a. Investigations on chilled beef. I. Microbial contamination acquired in the meat works. *Australian J. Council Sci. Ind. Research, Bulletin* No. 126.

- Empey, W. A. and W. J. Scott. 1939b. Investigations on chilled beef. II. Cooling and storage in the meat works. Australian Council Sci. and Ind. Research, Bulletin No. 129.
- Felsenfield, O., V. M. Young, and T. Yoshimura. 1950. A survey of *Salmonella* organisms in market meat, eggs and milk. J. Amer. Vet. Med. Assoc. 116: 17-21.
- Fine, J., R. Rutenburg, and F. B. Schweinburg. 1959. The role of the reticuloendothelial system in hemorrhagic shock. J. Exptl. Med. 110: 547.
- Floyd, T. M., J. R. Baronski, and M. El-gannani. 1953. Recovery of human enteric pathogens on meat from butcher shops in Cairo, Egypt. J. Infect. Dis. 92: 224-227.
- Flotz, V. D. 1941. A bacteriological study of ground meat. J. Bacteriol. 42: 289.
- Food Engineering Staff. 1962. Humane slaughter. Food Eng. 34: 2, 52.
- Food Protection Committee. 1964. An evaluation of public health hazards from microbiological contamination of foods. National Academy of Sciences-National Research Council, Washington, D. C. Publication No. 1195.
- Ford, W. W. 1901. On the bacteriology of normal organs. J. Hygiene. 1: 277.
- Frank, E. D., J. B. MacDonald, C. Palmerio, F. B. Schweinburg, and J. Fine. 1961. Effect of hemorrhagic shock on viability of invading bacteria. Proc. Soc. Exptl. Biol. Med. 106:394.
- Frank, H. A. 1963. Factors affecting bacterial spoilage of animal products at elevated temperatures. Food Technol. 17: 573-574.
- Frazier, W. C. 1967. Food Microbiology. 2nd ed. p. 252-282. McGraw-Hill Book Company, Inc., New York.
- Galton, M. M., W. Lowery, and A. V. Hardy. 1954a. Salmonella in fresh and smoked pork sausage. J. Infect. Dis. 95: 232-235.

- Galton, M. M., W. V. Smith, H. B. McElroth, and A. V. Hardy. 1954b. Salmonella in swine and cattle and the environment of abattoirs. J. Infect. Dis. 95: 236-245.
- Galton, M. M. and J. H. Steele. 1961. Laboratory and epidemiological aspects of foodborne diseases. J. Milk and Food Technol. 24: 104-114.
- Galazka, H. 1969. Home-killed meat. Agriculture. 76: 224-227.
- Gardner, G. A. and A. W. Carson. 1967. Relationship between carbon dioxide production and growth of pure strains of bacteria on porcine muscle. J. Appl. Bacteriol. 30:500-510.
- Geister, R. S. and A. C. Maack. 1967. Sanitation in meat and poultry processing plants. J. Milk and Food Technol. 30: 67-70.
- Glage, F. 1901. Ueber die Bedeutung der Aromabakterien fur die Fleischhygiene. Z. Fleisch-u. Milchhyg. 11: 131.
- Goldberg, H. S. 1952. Studies on the microflora in deep tissues of beef and their susceptibility to various antibiotics. Bacteriol. Proc. 17.
- Goldberg, H. S., H. H. Weiser, and F. E. Deatherage. 1953. Studies on meat. IV. The use of antibiotics in preservation of fresh beef. Food Technol. 7: 4, 165-166.
- Gomutputra, C. and F. W. Fabian. 1953. Acids and chloroamphenicol as sanitizing agents for meat contaminated with food poisoning organisms. J. Milk and Food Technol. 16: 220-227.
- Graner, M., V. R. Cahill, and H. W. Ockerman. 1969. The emulsifying capacity of bovine muscle tissue as related to time post-mortem and Microbial contamination. Food Technol. 23: 94-97.
- Greenberg, R. A., R. B. Tompkin, B. O. Bladel, R. S. Kittaka, and A. Anellis. 1966b. Incidence of mesophilic Clostridium spores in raw pork, beef, and chicken in processing plants in the United States and Canada. Appl. Microbiol. 14: 789-793.
- Greer, L. P., W. T. Murry, and E. Smith. 1933. Bacterial content of frosted hamburger steak. A. J. of Pub. Health 23: 673.

- Gustin, P. N., and R. H. Siddiqi. 1970. Bacterial contamination of the hog carcass during slaughter and dressing procedures. Unpublished, Personal Communication.
- Haines, R. B. 1930. Dept. Sci. Ind. Res. Food Invest. Board Spec. Rept. No. 36, London, England. (cited by Callow, E. H. 1952).
- Haines, R. B. 1931a. The growth of microorganisms on chilled and frozen meat. J. Soc. Chem. Ind. 50: 223.
- Haines, R. B. 1931b. Influence of temperature on rate of growth of Sporotrichum carnes from -10°C to 3°C. J. Exp. Biol. 8: 379-388.
- Haines, R. B. 1932. Influence of temperature on rate of growth of saprophytic Actinomyces. J. Exp. Biol. 9: 45-60.
- Haines, R. B. 1933a. Observations on the bacterial flora of some slaughterhouses. J. Hyg. 33: 165.
- Haines, R. B. 1933b. The bacterial flora developing on stored lean meat, especially with regard to "slimy" meat. J. Hyg. (Gr. Brit.). 33: 175.
- Haines, R. B., and E. C. Smith. 1933. The storage of meat in small refrigerators. Dept. Sci. Ind. Res. Food Invest. Board (Brit.), Special Rept. No. 43.
- Haines, R. B. 1934. The minimum temperatures of growth of some bacteria. J. Hygiene. 34: 277.
- Haines, R. B. 1937. Microbiology in the preservation of animal tissues. Gt. Britain Dep. Sci., Ind. Res., Food Investigation. Spec. Rept. No. 45.
- Haines, R. B. 1938. The effects of freezing on bacteria. Proc. Roy. Soc. (B) 124: 451-463.
- Haines, R. B. and W. J. Scott. 1940. Anaerobic organism associated with "bone taint" in beef. J. Hyg. 40: 154.
- Hall, H. E. and R. Angelotti. 1965. Clostridium perfringens in meat and meat products. Appl. Microbiol. 13: 352-357.

- Halleck, F. E., C. O. Ball and E. F. Stier. 1958a. Factors affecting quality of prepackaged meat. IV. Microbial studies. A. Cultural studies on bacterial flora of fresh meat, classification by genera. Food Technol. 12: 197.
- Halleck, F. E., C. O. Ball and E. F. Stier. 1958b. Factors affecting quality of prepackaged meat. IV. Microbiological studies. B. Effects of package characteristics and of atmospheric pressure in package upon bacterial flora of meat. Food Technol. 12: 301.
- Halleck, F. E., C. O. Ball, and E. F. Stier. 1958c. Factors affecting quality of prepackaged meat. IV. Microbial studies. C. Effects of initial bacteria count, kind of meat, storage time, storage temperature, antioxidants, and antibiotics on the rate of bacterial growth in packaged meat. Food Technol. 12: 654-659.
- Hannan, R. S. 1962. Some properties of packaging materials which relate to the microflora of the contents. J. Appl. Bact. 25: 248.
- Hanson, R. E., R. Rogers, S. Emse, and N. J. Jacobs. 1964. Incidence of Salmonella in the hog colon as affected by handling practices prior to slaughter. J. Amer. Vet. Med. Assoc. 145: 139-140.
- Harriman, L. A., V. J. Del Giudice, B. M. Shinn, and R. Hansen. 1948. The incidence of putrefactive anaerobes in pork, Illinois Bacteriologists, Fall Meeting. Peoria, Ill. Oct. 15, 1948.
- Horwood, M. P. and V. A. Minch. 1951. The number and types of bacteria found on the hands of food handlers. Food Res. 16: 133.
- Houthuis, M. J. J. 1957. Transportation, ante-mortem care, and inspection of animals intended for slaughter. In: Meat Hygiene. WHO nomograph series No. 33. p. 111-122.
- Healthy Animals, Wholesome Meat. 1962. Ag. Res. 11: 4-5.
- Hoestra, J. 1955. Durability of Meat. Am. Vet. Med. Assoc. J. 126: 238.

- Hoffstadt, R. E. 1924. The bacteriological examination of ground beef. *Am. J. Hyg.* 4: 33.
- Hulphers, G. 1933. Skallvatten floran i Svinlungen. *Nordiska Veterinärmötet*. p. 800-813. Helsingfors, Finland. (cited by Ayres 1955).
- Ingraham, J. L. 1958. Growth of psychrophilic bacteria. *J. Bacteriol.* 76: 75.
- Ingram, M. 1935. The microbiology of pork and bacon. (Gr. Brit.) *Dept. Sci. Ind. Research, Rep. Food Invest. Board, No. 77.*
- Ingram, M. 1949. Hygiene and Storage. *J. Roy. Sanit. Inst.* 69:39.
- Ingram, M. 1962. Microbiological principles in prepackaging meats. *J. Appl. Bacteriol.* 25: 259-281.
- Jay, J. M. 1961. Incidence and properties of coagulase-positive Staphylococci in certain market meats as determined on 3 selective media. *Appl. Microbiol.* 9: 228-232.
- Jay, J. M. 1962. Further studies on Staphylococci in meat. III. Occurrence and characteristics of coagulase-positive strains from a variety of nonfrozen market cuts. *Appl. Microbiol.* 10: 247-251.
- Jay, J. M. 1964a. Release of aqueous extracts by beef homogenates, and factors affecting release volume. *Food Technol.* 18: 129.
- Jay, J. M. 1964b. Beef microbial quality determined by extract-release (ERV). *Food Technol.* 18: 133.
- Jay, J. M. 1964c. Comparison of the new ERV test for detecting spoilage in beef with several other indices of microbial quality in meats. *Bact. Proc. American Society for Microbiology.*
- Jay, J. M. 1965. Relationship between water holding capacity of meats and microbial quality. *Appl. Microbiol.* 13: 120.
- Jay, J. M. 1966a. Response of the extract-release volume and water holding capacity phenomenon to microbiologically spoiled beef and aged beef. *Appl. Microbiol.* 14: 492-496.

- Jay, J. M. 1966b. Influence of post-mortem conditions on muscle microbiology. In: The Physiology and Biochemistry of Muscle as a Food. The University of Wisconsin Press. p. 387-402.
- Jaye, M., R. S. Kittaka, and Z. J. Ordal. 1962. The effect of storage and packaging material on the storage life and bacterial flora of ground beef. Food Technol. 16: 4, 95-98.
- Jensen, L. B., and W. R. Hess. 1941. A study of ham souring. Food Res. 6: 273.
- Jensen, L. B. 1944. Microbiological problems in the preservation of meat. Bacteriol. Revs. 8: 161.
- Jensen, L. B. 1954. Microbiology of Meats. 3rd. ed. Garrard Press, Champaign, Ill.
- Jepsen, A. 1947. Bacteriology of pork. Microbial contamination acquired in the meatworks Royal Veterinary and Agricultural College, Copenhagen, Yearbook, 40.
- Jepsen, A. 1957. Application of bacteriological and biochemical tests in the hygienic judgement of meat and meat products. In: Meat Hygiene. WHO Monograph series No. 33, p. 235-250.
- Kaplan, M. M. 1957. Meat-hygiene problems in tropical areas. In: Meat Hygiene. WHO Monograph Series No. 33, Geneva.
- Kefford, J. F. 1948. Effect of ozone on microbial growth on beef muscle, and on the flavor of beef fat. (Australia) J. Council Sci. Ind. Research. 21: 116-140.
- Khan, M., and M. Katamay. 1969. Antagonistic effect of fatty acids against Salmonella in meat and bone meal. Appl. Microbiol. 17: 402-404.
- Kirsch, R. H., F. F. Berry, C. L. Baldwin, and E. M. Foster. 1952. The bacteriology of refrigerated ground beef. Food Res. 17: 495.
- Kitchell, A. G. 1962. Micrococci and coagulase negative staphylococci in cured meats and meat products. J. Appl. Bacteriol. 25: 3, 416-431.

- Kraft, A. A. and J. C. Ayres. 1952. Post-mortem changes in stored meats. IV. Effect of packaging materials on keeping quality of self-service meats. Food Technol. 6: 8-12.
- Kraft, A. A. and J. C. Ayres. 1954. Effect on display case lighting on color and bacterial growth on packaged fresh beef. Food Technol. 8: 290-295.
- Kraybill, H. R. 1951. New developments in meat products. Food Technol. 5: 485-487.
- Lawrie, R. A. 1966. Meat Science. Pergamon Press Ltd. New York. p. 142-177.
- Le Fevre, E. A. 1917. A bacteriological study of hamburger steak. Am. Food J. 12: 140.
- Lepovetsky, B. C., H. H. Weiser, and F. E. Deatherage. 1953. A microbiological study of lymph nodes, bone marrow, and muscle tissue obtained from slaughtered cattle. Appl. Microbiol. 1: 57.
- Libby, J. A. 1967. The controversial cause of sour beef. (original article). Meat Hygiene Abstracts. 8: 1, 1-4.
- Mallman, W. L., and L. Zaikowski. 1940. Microbiological studies on bacteria isolated from cold storage meat and meat products. I. Identification. II. Effects of carbon dioxide on growth. (abstract) Inst. Food Technol. Food Conf. Proceedings. 1: 385.
- Manual of Meat Inspection Procedures of the United States Dept. of Agriculture, 1968.
- Marxer A. 1903. Beitrage zur Frage Bakteriengehalts und der Haltbarkeith des Fleisches. (as reviewed by Weinzirl 1924).
- Massee, G. 1912. On the discolored spots sometimes present on chilled beef, with special reference to "black spot". J. Hygiene. 12 : 489.
- McCoy, D. W. and J. E. Faber, 1966. Influence of food micro-organisms on Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 14: 372-377.

- McCullough, N. B., C. W. Eisele, and E. Pavelchek. 1951a.
Survey of brucellosis in slaughtered hogs. Pub. Health
Repts. (U.S.). 66: 205.
- McCullough, N. B., C. W. Eisele, and A. F. Byrne. 1951b.
Incidence and distribution of Brucella abortus in
slaughtered Bang's reactor cattle. Pub. Health Repts.
(U.S.) 66: 341.
- McLean, R. A., and W. L. Sulzbacher. 1953. Microbacterium
thermosphactum, spec nov; a nonheat resistant bacterium
from fresh pork sausage. J. Bact. 65: 428.
- Medrek, T. F. and E. M. Barnes. 1962. The distribution of group
D streptococci in cattle and sheep. J. Appl. Bact. 25:
159-168.
- Melrose, D. R. 1970. Present and future veterinary work con-
cerning meat production. Vet. Rec. 86: 74-75.
- Mercure, M. 1967. Interpretation of bacterial count in relation
to the quality of meat. Canad. J. Public Health. 58:
457-460.
- Miller, W. A. 1955. Effect of freezing ground pork and subse-
quent storing above 32°F upon the bacterial flora. Food
Technol. 9: 332-334.
- Miller, W. A. 1964. The microbiology of self-service, packaged
fresh pork sausage. 27: 1-3.
- Miller, W. A. 1967. Bacteria and yeast counts of prepackaged,
sliced, cured bacon, and sliced, fresh side pork. J. Milk
and Food Technol. 30: 2, 36-38.
- Moran, T., and E. C. Smith. 1929. Post-mortem changes in animal
tissues. The conditioning or ripening of beef. Dept. Sci.
Ind. Research Food Invest. Board. (Brit.) Special Rept.
No. 36.
- Moran, T., E. C. Smith, and R. G. Tomkins. 1932. The inhibition
of mold growth on meat by carbon dioxide. J. Soc. Chem.
Ind. 51: 114.
- Moran, T. 1935. Post-mortem and refrigeration changes in meat.
J. Soc. Chem. Ind. 54: 149T-151T.

- Moorehouse, E. C., M. O'Grady and H. O'Connor. 1970. Contamination of meat carcasses. *Lancet*. 2: 664.
- Muftic, A. and A. H. Benganovic. 1965. On the biochemical activity of bacteria in the process of decomposition of meat. *Meat Hygiene Abstracts*. 6: 2, 20.
- National Academy of Sciences. 1964. An Evaluation of Public Health Hazards from Microbial Contamination of Foods. Washington, D. C. Pub. No. 1195. p. 16-17, 22.
- Niven, C. F., A. G. Castellani, and V. A. Allanson. 1949. Study of the lactic acid bacteria that causes surface discoloration of sausage. *J. Bacteriol.* 58: 633.
- Niven, C. F., Jr. 1951. Influence of microbes upon the color of meats. *American Meat Inst. Foundation Circular No. 2*.
- Niven, C. F., Jr. 1957. A perspective of the sanitary problems in the meat industry. *American Meat Institute Foundation. Circular No. 39*. p. 1-9.
- Niven, C. F., Jr. 1961. *Microbiology of Meats*. American Meat Inst. Found. Circular No. 68.
- Nottingham, P. M. 1960. Bone-taint in beef: II. Bacteria in ischiatic lymph nodes. *J. Sci. Food Agri.* 11: 436-441.
- Ockerman, H. W., V. R. Cahill, K. E. Davis, and C. E. Davis. 1964. Tissue storage without contaminants. *J. Am. Sci.* 23: 142-146.
- Ockerman, H. W. and V. R. Cahill. 1967. How to get bacteria-free meat samples. *The National Provisioner*. 156: 10, 35.
- Ockerman, H. W. 1969. Microbiological contamination found on porcine skin during the slaughter operation. Unpublished.
- Ockerman, H. W., V. R. Cahill, H. H. Weiser, C. E. Davis, and J. R. Siefker. 1969. Comparison of sterile and inoculated beef tissue. *J. Food Sci.* 34: 93-99.

- Ogilvy, W. S., and J. C. Ayres. 1951. Post-mortem changes in stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging storage life of cut-up chicken. *Food Technol.* 5: 97.
- O'Neill, R. D. 1964. The microbiological significance of food packaging materials. *J. Milk and Food Tech.* 27: 4, 116-119.
- Ordal, Z. J. 1962. Anaerobic packaging of fresh meat. *Proc. 14th Res. Conf. American Meat Inst. Foundation. Circular No. 70.* p. 39-45.
- Patterson, J. T. 1967. Hygiene in meat processing plants 1.- Importance of bacteria in meat processing plants. *Rec. agric. Res. Minist. Agric. Nth. Ir.* 16: 13-18.
- Patterson, J. T. 1968a. Hygiene in Meat processing plants 2.- Methods of assessing carcass contamination. *Rec. agric. Res. Minist. Agric. Nth. Ir.* 17: 1-5.
- Patterson, J. T. 1968b. Hygiene in meat processing plants 3.- Methods of reducing carcass contamination. *Rec. agric. Res. Minist. Agric. Nth. Ir.* 17: 7-12.
- Patterson, J. T. 1969. Meat hygiene. II. Hygiene during slaughter and subsequent treatment of the carcass. *Vet. Rec.* 85: 536-541.
- Pearson, D. 1968. Assessment of meat freshness in quality control employing chemical techniques: A review. *J. Sci. Food and Agri.* 19: 357-363.
- Post, F. J. and J. L. Balzer. 1963. Effect of hexachlorophene detergent on the microbial population of the hands of food handlers. *J. of Milk and Food Tech.* 26: 5, 142.
- Potter, C. W., J. R. Archer, G. C. Shild. 1968. *Introduction to Medical Microbiology.* New York. Appleton-Century-Crofts. p. 170-174.
- Prescott, S. C., J. F. Hale, and C. E. White. 1931. Observations on the bacteriology of slimy beef. *J. of Bacteriol.* 21: 26.
- Price, P. B. 1938. New studies in surgical bacteriology and surgical technique. *J. Amer. Med. Assn.* 111: 1993.

- Ramsbottom, J. M. 1954. Meat packaging criteria. *Modern Packaging*. 27: 6, 131-134.
- Reith, A. F. 1926. Bacteria in the muscular tissues and blood of apparently normal animals. *J. Bacteriol.* 12: 367-383.
- Retrum, R. 1957. Beef carcass chilling holding. *Am. Soc. Refrig. Eng. Refrigeration and Meat Packing Conference*.
- Rey, C. R., A. A. Kraft, H. W. Walker, and F. C. Parrish, Jr. 1970. Microbial changes in meat during aging at elevated temperature and later refrigerated storage. *Food Technol.* 24: 67-71.
- Rice, E. E., J. F. Fried, and W. R. Hess. 1946. Storage and microbial action upon vitamins of the B-complex in pork. *Food Res.* 11: 305.
- Richardson, J. H., V. J. Del Guidice, and C. K. Wiesman. 1954. Hazards of spores in floorcoverings-sawdust. *Appl. Microbiol.* 2: 177.
- Riemann, 1965. Bacteriological meat inspection. (Univ. of Calif., Davis, Calif.) A Paper. 1-15, 1964. Abstracted from *Meat Hygiene Abstracts*. 6: 4, 21-23.
- Robinson, R. H. M., M. Ingram, R. A. M. Case and J. G. Benstead. 1953. Whalemeat: Bacteriology and hygiene. *Dept. Sci. Indust. Res. Food Invest. Spec. Rept. No. 58*, p. 47.
- Robinson, R. H. M., M. Ingram, R. A. M. Case and J. G. Benstead. 1953. Whalemeat: Bacteriology and hygiene. *Spe. Rept. Fd. Invest. Bd. London. No. 59*, p. 56.
- Rogers, R. E. and C. S. McClesky. 1957. Bacteriological quality of ground beef in retail markets. *Food Technol.* 11: 318.
- Ross, A. I. 1958. Hygienic preparation of meat products. *Food Manufacture*. 273-278.
- Ruben, H. L., M. Scherago, and R. H. Weaver. 1942. The occurrence of Salmonella in the lymph glands of normal hogs. *Amer. J. Hyg.* 36: 43-47.
- Sadler, W. W. 1963. Public health aspects of handling animal products in the tropics. *Food Technol.* 17: 154-156.

- Saffle, R. L., K. N. May, H. A. Hamid, and J. D. Irby. 1961. Comparing three rapid methods of detecting spoilage in meat. *Food Technol.* 15: 465.
- Sanborn, W. R. 1963. The relation of surface contamination to the transmission of disease. *Am. J. of Pub. Health.* 53: 8, 1278-1283.
- Sanitation Handbook of Consumer Protection Programs. 1968. U.S.D.A., C. & M.S.
- Satterlee, L. D. and A. A. Kraft. 1969. Effect of meat and isolated meat proteins on the thermal inactivation of Staphylococcal enterotoxin B. *App. Microbiol.* 17: 906-909.
- Scarafoni, G. S. 1957. Hygienic construction and technical organization of slaughterhouses. In: *Meat Hygiene*. WHO Monograph Series No. 33, p. 125-136.
- Schmidt, W. 1931. Einfluss von temperatur und feuchtigkeit auf das bakterienwachstum auf gekuhltem fleisch. *Beih. Z. ges. Kalteindustr.*, Reihe 3, Heft 6.
- Schönberg, F. 1957. Hygienic aspects of meat processing. In: *Meat Hygiene*. WHO Monograph Series No. 33, p. 253-262.
- Scott, W. J. 1931. The council for scientific and industrial research, meat investigations, Parliament of the Commonwealth of Australia, pp. 61.
- Scott, W. J. 1936. The growth of microorganisms on ox muscle. I. The influence of water content of substrate on rate of growth at -1°C . *J. Council Sci. Ind. Research.* 9: 177.
- Sharp, J. G. 1963. Aseptic autolysis in rabbit and bovine muscle during storage at 37° . *J. Sci. Fd. Agric.* 14: 468-479.
- Sharpe, E. M. 1962. Lactobacilli in meat products. *Food Manufacture.* 37: 582-589.
- Shelef, L. A. and J. M. Jay. 1969. Relationship between amino sugars and meat microbial quality. *Appl. Microbiol.* 17: 931-932.

- Silliker, J. H., H. P. Andrews, and J. F. Murphy, 1957. New non-destructive method for the bacteriological sampling of meats. *Food Technol.* 11: 317-318.
- Silliker, J. H., J. L. Shank, and H. P. Andrews. 1958. Simultaneous determination of total count and fluorescent pseudomonads in fresh meat and poultry. *Food Technol.* 12: 255-257.
- Sleeth, R. B., R. L. Hendrickson, and D. E. Brady. 1957. Effects on controlling environmental conditions during aging on the quality of beef. *Food Technol.* 11: 205.
- Sleeth, R. B., G. G. Kelley, and D. E. Brady. 1958. Shrinkage and organoleptic characteristics of beef aged in controlled environments. *Food Technol.* 12: 86.
- Smith, D. T. and D. S. Martin. 1948. *Textbook of Bacteriology*. 9th Ed. Appleton-Century-Croft, Incorporated.
- Smith, H. W., and W. E. Crabb. 1961. The bacterial flora of the feces of animals and man; its development in the young. *J. Path. Bact.* 82: 53-66.
- Smith, H. G. M. 1964. Salmonellae in abattoirs, butchers shops and home produced meats, and their relation to human infection. *J. Hyg. (London)* 62: 282-302.
- Spray, R. S. 1922. The bacteria in normal and diseased lungs of swine. *J. Infect. Dis.* 31: 10.
- Standfuss, R. 1952. Bacteriological Meat Inspection. *Amer. Vet. Med. Assoc.* 121: 111.
- Stuart, C. A., E. Van Stratum, and R. Rustigian. 1945. Further studies on urease production by Proteus and related organisms. *J. Bact.* 49: 437.
- Straka, R. P. and J. L. Stokes. 1960. Psychrophilic bacteria from Antarctica. *J. Bacteriol.* 80: 622.
- Steinkraus, K. H. and J. C. Ayres. 1964a. Incidence of putrefactive anaerobic spores in meat. *J. Food Sci.* 29: 87-93.

- Steinkraus, K. H. and J. C. Ayres. 1964b. Biochemical and serological relationships of putrefactive anaerobic spore-forming rods isolated from pork. *J. Food Sci.* 29: 100-104.
- Stringer, W. C. and H. D. Haumann. 1966. Influence of pre-display temperature and muscle firmness on microflora and sensory attributes of prepackaged beef. *Food Technol.* 20: 108.
- Stringer, W. C., M. E. Bilskie, and H. D. Haumann. 1969. Microbial profiles in fresh beef. *Food Technol.* 23: 97-102.
- Sulzbacher, W. L. 1950. Survival of microorganisms in frozen meat. *Food Technol.* 4: 386-390.
- Sulzbacher, W. L. and R. A. McLean. 1951. The bacterial flora of fresh pork sausage. *Food Technol.* 5: 7-8.
- Sulzbacher, W. L. 1952. Effect of freezing and thawing on the growth rate of bacteria in ground meat. *Food Technol.* 6: 341.
- Sulzbacher, W. L. 1953. Report of subcommittee on microbiological methods of meat research. *Proc. 5th Annual Reciprocal Meat Conference. National Livestock and Meat Board, Chicago.* 170.
- Tissier and Martelly. 1902. Recherches sur la putrefaction de la viande de boucherie. *Ann. Inst. Past.* 16: 865-903.
- Tobey, E. R. 1944. Analyses of hamburger steak. *Maine Agr. Exp. Sta., Offic. Insp. Bull. No. 191*, p. 145-147. *Chemical Abs.*, 8363q (1948).
- Thornton, H. 1962. *Textbook of meat inspection.* 4th ed. London.
- Tomkins, R. G. 1932. The inhibition of the growth of meat-attacking fungi by carbon dioxide. *J. Soc. Chem. Ind.* 51: 261.
- Umbreit, W. W. 1962. *Modern Microbiology.* W. H. Freeman and Company, San Francisco and London. p. 327-330.

- Vanderzant, C. and R. Nickelson. 1969. A microbiological examination of muscle tissue of beef, pork, and lamb carcasses. J. of Milk and Food Technol. 32: 10, 357-361.
- Vickery, J. R. 1936a. The action of microorganisms on fat. I. The hydrolysis of beef fat by some bacteria and yeast tolerating low temperatures. J. Council Sci. Ind. Research. 9: 107.
- Vickery, J. R. 1936b. The action of microorganisms on fat. II. A note on the lipolytic activities of further strains of microorganisms tolerating low temperatures. J. Council Sci. Ind. Research. 9: 196.
- Voegeli, M. M., L. J. Bratzler and W. L. Mallman. 1953. Flow sheets of prepackaged fresh meats. Food Technol. 7: 350-353.
- Warnecke, M. O., H. W. Ockerman, H. H. Weiser, and V. R. Cahill. 1966. Quality of processed comminuted meats as affected by microbial flora of the raw constituents. Food Technol. 20: 686-688.
- Warnecke, M. O., J. A. Carpenter, and R. L. Saffle. 1967. A Study of Clostridium botulinum Type E. in vacuum packed meat. Food Tech. 21: 115-116.
- Weinzirl, J. and E. B. Newton. 1914a. Bacteriological methods for meat analysis. Am. J. Pub. Health. 4: 408-412.
- Weinzirl, J. and E. B. Newton. 1914b. Bacteriological analysis of hamburger steak with reference to sanitary standards. Am. J. Public Health. 4: 413.
- Weinzirl, J. and E. B. Newton. 1915. The fate of bacteria in frozen meat held in cold storage and its bearing on a bacteriological standard for condemnation. Am. J. Public Health. 5: 833-835.
- Weinzirl, J. 1924. Concerning the relation of the bacterial count to the putrefaction of meat. Am. J. Public Health. 14: 946.
- Weiser, H. H., L. E. Kunkle, and F. E. Deatherage. 1954. The use of antibiotics in meat processing. Appl. Microbiol. 2: 88.

- Weissman, M. A., and J. A. Carpenter. 1969. Incidence of salmonella in meat and meat products. Appl. Microbiol. 17: 899-902.
- Williams, L. P. and K. W. Newell. 1967. Patterns of Salmonella excretion in market swine. Am. J. Public Health. 57: 466-471.
- Williams, M. L. 1967. A new method for evaluating surface contamination of raw meat. J. Appl. Bacteriol. 30: 498-499.
- Wolin, E. F., J. B. Evans, and C. F. Niven. 1957. The microbiology of fresh and irradiated beef. Food Res. 22: 682.
- Wright, A. M. 1912. Chemical and bacteriological study of fresh and frozen New Zealand lamb and mutton. J. Soc. Chem. Ind. 31: 965-967.
- Wright, A. M. 1923. Moulds on frozen meat. J. Soc. Chem. Ind. 42: 488T-490T.
- Zender, R., C. Lataste-Dorolle, R. A. Collet, P. Rowinski, and R. F. Mouton, 1958. Aseptic autolysis of muscle: Biochemical and microscopic modification occurring rabbit and lamb during aseptic and anaerobic storage. Food Res. 23: 305.
- Ziegler, P. T. 1952. Meat We Eat. 3rd. Ed. The Interstate Publishers.

A REVIEW OF THE MICROBIOLOGY OF FRESH MEAT

by

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Spoilage or reduced keeping time of meat has been shown largely to be attributed to growth of microorganisms especially bacteria, and the subsequent release of their metabolic products.

The aim of this paper has been to present a literature review on how fresh meat becomes contaminated by microorganisms, and how these microorganisms grow on the meat.

The initial invasion of meat by microorganisms begins with the slaughtering operations. The first and most important source appears to be the animal itself. Enormous numbers of organisms are associated with the hide, the gastrointestinal tract, and with excretions voided by the animal. Other sources arise from the environment of the plant such as airborne contamination, aqueous sources, instruments used in the slaughtering and dressing operation, various vessels and receptacles and the personnel.

The number of bacteria increase as time in storage increases. Four principle bacterial genera have been shown to be found regularly from a large number of isolates during chilling and storage. These are Pseudomonas, Achromobacter, Flavobacterium and Micrococcus, Pseudomonas being the most prevalent genus found.

Of the mold genera found, the most common isolate has been Penicillium. Cladosporium, Thamnidium, Mucor and Rhizopus were next in order of prevalence.

Three genera of false yeasts -- Torulopsis, Candida and Rhodotorula are occasionally found; no true yeasts have been reported.

It is generally agreed that the onset of sliminess and off-odors is imminent when the number of bacteria per sq. cm. is in the order of 10^7 to 10^8 . The reason for this type of spoilage, rather than putrefaction has been attributed to the fact that though many bacteria capable of causing putrefaction do come in contact with the meat, they are mesophiles which do not survive refrigeration or grow poorly at low temperatures.

The time necessary for slime production is greatly influenced by the initial contamination. If this is high, then spoilage is rapid.

Contamination during the chilling, storage and shipping periods and further operations such as cutting, comminuting and packaging apparently are derived from the same environmental sources as during the dressing operations, (e.g., workmen, air, equipment, sawdust, floors and walls).