ACIDS AND CHLORAMPHENICOL AS SANITIZING AGENTS FOR MEAT CONTAMINATED WITH FOOD POISONING ORGANISMS

By

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A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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ABSTRACT

Food poisonings due to staphylococci and salmonellae are world-wide in distribution, especially when Salmonella typhosa which is the etiological organism of the typhoid fever is included in the Salmonella genus. Pork and beef have been found to be contaminated with these organisms and a survey in Illinois showed that as much as 0.2 per cent of the market beef and 14.3 per cent of the market pork yielded salmonellae on bacteriological examination.

A study was made to determine the difference in the amount of growth of the food poisoning organisms on the surface of raw lean pork and beef taken 1 cm beneath the surface of retail cuts, on the autoclaved lean pork and beef, and on the surface of regular retail cuts of raw lean pork and beef. The results showed that the growth was less on the surface of retail cuts of the meats due to antagonism offered by other bacteria and to desiccation.

Raw lean pork and beef were found to be good media for the growth of food poisoning staphylococci and salmonellae. Experimental incubation of the inoculated meats at various temperature ranges showed that the organisms increased to large numbers at 15.6-32.2 C (60-90 F).

The pork rind and the external fat covering the beef carcass were found to be poor media for the growth of the organisms at various temperature ranges.

Experiments were performed to compare the germicidal effect of trichloracetic acid, dichloracetic acid, dehydroacetic acid (sodium salt),

monochloracetic adid, acetic acid, and chloramphenicol on the food poisoning organisms on lean pork and beef. The results showed that acetic acid was more germicidal than the other acids in 1 per cent solution and than 10 ug/ml chloramphenicol.

Four per cent vinegar was found to be a practical and more effective dipping solution than 1 per cent acetic acid in destroying the food poisoning organisms on the pork and beef. The solution did not impart any appreciable flavor to the meats.

A method of surface plating to determine the number of staphylococci and salmonellae on a selective and a differential medium respectively is described.

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INTRODUCTION

While food poisoning due to staphylococci is considered to comprise about some ninety per cent of all food poisoning cases in North America, Dolman (27) points out that food poisoning outbreaks are notorious for unreliability of statistics respecting their incidence.

Inasmuch as many countries do not possess cheap and readily available electricity and refrigerating equipment, the incidence of food poisoning should be higher, especially in tropical regions where the temperature is nearer to the optimum for food poisoning bacteria all the year round, than it is in this country. Many countries in the Far East usually have the meat slaughtered early in the day and sold as fresh meat up to 24 hours for beef, and during a shorter time for pork depending on the season of the year. Consequently much sickness undoubtedly is due to consumption of contaminated meat.

As will be pointed out later, market meat and meat products in the United States are by no means free of food poisoning bacteria such as salmonellae, and, naturally, staphylococci which are ubiquitous. Furthermore a certain amount of meat is consumed which has not been inspected by a trained veterinarian, and, also, wild game which can not be immediately refrigerated.

It is the purpose of this investigation to determine the viability of some food poisoning staphylococci and salmonellae on the surface of beef and pork at different temperatures, and also to study the influence

of treating the surface of the meat with certain common germicides. The results should demonstrate the effects of refrigeration,of keeping meats at various temperature ranges, and also of treatment with various agents on the numbers of viable bacteria on contaminated meat.

LITERATURE REVIEW

General Consideration

According to Tanner (89), when meat was inoculated with pure culture of bacteria which might be expected to be present in such a product, its reaction was rendered more alkaline. A reaction of pH 6.0 to 6.2 may indicate incipient spoilage, but it is doubtful whether pH methods can be used for determining the fitness of meat. The pH of meat depends on too many variable factors, such as autolysis, rapidity of chilling, etc. Furthermore, for some people, changes in the meat which would alter the pH significantly would be considered desirable. For these reasons, slight changes in pH could not be the only method used for judging the quality of meat.

Tanner (89) stated "Since spoilage of tissues, apart from special cases of anaerobic processes, is due to growth on the surface of the carcass, the best procedure, according to Haines, is to make counts per unit of superficial tissue. His method for this was as follows: A sterile cork borer is pressed vertically into the tissues to a depth of one centimeter or more. By this means a cylinder of tissue is left, and from its top a disc about two millimeters thick is cut with sterile scalpel. The area of this disc may be computed from the diameter of the cork borer. Microorganisms on the surface of the sample may be brought into suspension by shaking vigorously with glass beads and sterile salt solution. When it is believed that bacteria may have penetrated, a thicker disc of meat may be taken and ground, or disintegrated with a

mechanical stirrer and sterile sand." "Bacteriological methods probably have distinct limitations in routine meat examination on account of the time consumed and the fact that meat may acquire many bacteria after preparation for the market." "Organoleptic tests, largely used for fresh meats and sea foods, leave much to be desired."

Weaver (95) designed a test which showed the increase in activity of hydrogen sulfide producing organisms in hamburger steak during the period of incipient putrefaction. It was possible by using this test to detect putrefaction before it could be demonstrated by organoleptic methods. The method of testing was stated as follows: "Place one gram of meat in a tube containing exactly 10 cc of standard infusion or extract broth. Suspend a strip of lead acetate paper beside the cotton plug. Incubate at 37°C in an anaerobic jar under partial vacuum. Examine and record the number of hours necessary to obtain blackening of the acetate paper due to the production of hydrogen sulfide." He found that comparatively good hamburger steak gave positive tests in seven to ten hours. At the time when putrefaction became evident by the production of a foul odor, positive tests were obtained in two to five hours. He also mentioned that hamburger steak which did not give a positive test in six hours would keep at least three days in a good refrigerator.

It was found (89) by experiment with lamb and mutton that when kept at temperature of 60-77° F the surface bacteria could invade the interior of the lamb in five days and the interior of the mutton in seven days, but no bacterial growth or invasion took place at temperature of 2-19° F.

The two most important factors which influence development of bacteria on meat surfaces probably are temperature and humidity (89). Growth of bacteria on meat surface ceases at relative humidities below 92 per cent, and bacteria grow with great rapidity at the highest attainable humidity of 99 per cent. However some bacterial growth was observed at a relative humidity of 70 per cent in still air.

Segalove and Dack (80) found that growth of food poisoning strains of staphylococci isolated from food poisoning outbreaks was not evident in samples of dehydrated meat containing 20 per cent moisture or less. However they grew in all the meat samples containing 40 per cent or more moisture regardless of salt content up to 4.56 per cent. The S. enteritidis strain used by the investigators grew scantily in only one pork sample at the low salt concentration of 0.28 per cent adjusted to 50 per cent moisture. In meat samples adjusted to 60 per cent moisture, growth of S. enteritidis occurred in all but three samples of high salt contents from 2.20-3.70 per cent. The dehydrated samples were however autoclaved prior to inoculation.

Many bacteria were found on sides of fresh pork (89) prepared under laboratory conditions, with an average of 2×10^6 and 160,000 per sq. cm. respectively for two batches of pigs. Commercial sides of pork gave a mean count after chilling of 28,000, after curing of 60,000, and after maturation of 10^6 or more organisms per sq. cm.

Haines (38) found that lean beef held at temperatures of 0 ± 0.1 C ($32.0 \pm .18$ F) had an average bacterial count of 40,000 per square centimeter of surface area.

While various factors are involved in contributing microorganisms to beef, the chief source was the hide and hair of the slaughtered animals (89). The main contamination is bacterial, particularly at 68°F. At 30.2°F molds and yeasts may constitute 35 per cent of the initial microbial population on beef after slaughter, and at this temperature Achromobacter species have been found to constitute 90 per cent of the flora. The latter organisms may cause sliminess on meat and other species of bacteria may cause rancidity of fat under cold storage. The high percentage of cocci and bipolar organisms may indicate invasion from the respiratory tract and skin.

Jensen and Reeve (48) proved by series of experiments that the sticking operation in slaughtering hogs can introduce souring bacteria into the circulation, bones, and tissues. They used sterile knives, dipped separately into cultures of Clostridium sporogenes, Clostridium flabelliferum, Clostridium multifermentans, and Bacillus prodigiosus, to plung into the neck in the usual manner after the skin had been previously disinfected thoroughly. After the hogs were allowed to bleed in the rail for the standard time (12 minutes), then dropped into scalding vat and passed through all operations, including 24 hour chill, the bacteria could be recovered from various parts of the body, especially the bone marrow, in the majority of cases. They concluded that most primary bacterial contamination of pork results from this operation.

Brewer (4), in his work with the bacteriological content of market meats, concluded that:

1. Counts from different samples of the same cuts of meat usually varied more than those from different meats.

2. Prepared meats usually contained more bacteria than fresh meats.

3. Smoked meats contained a much smaller number of bacteria than fresh meats.

4. Of the bacteria encountered in the different meats, the colon group predominated.

5. The appearance of fresh meats could not be taken as an index to its bacterial content.

Bacteria develop quite slowly on beef at first (89). During this lag period they are developing on soluble nitrogenous substances already present on the beef. After a lag period of some 40 hours at 98.6°F they develop rapidly.

Staphylococcus food poisoning

Organisms responsible

Staphylococci isolated from food poisoning outbreaks were enterotoxin producing. Some of them were not identified as to species, while others were Staphylococcus aureus, Staphylococcus albus, and hemolytic staphylococci (19).

Staphylococcus pyogenes was suspected of being responsible for gastrointestinal disturbances in an outbreak (21), which resulted from ingesting the meat of a cow that died of puerperal fever.

Prior to 1930 the ability of staphylococci to elaborate a substance capable of provoking symptoms of food poisoning in human being was almost unsuspected. The "modern era" of Staphylococcus food poisoning, therefore, dates from 1930. Since that time our knowledge of the subject has increased rapidly, and this most common of all types of food poisoning is now recognized throughout the civilized world (18).

While there had been indications earlier that these organisms might be associated with gastrointestinal disturbances, in general, their presence in suspected foods was attributed to their ubiquitous nature.

Many filtrates from enterotoxigenic strains of staphylococci were tried on human volunteers and typical symptoms were produced (49,50).

Among foods involved in Staphylococcus food poisoning listed by Haynes and Hucker (39) are various kinds of pie and cake, usually custard filled, ice cream, butter, cheese, eclairs, cream puffs, beef, chicken, chicken gravy, chicken and ham paste, chicken salad, frozen

pack vegetables, ground meat sandwiches, ham, headcheese sandwiches, kippers, oysters, pastry, potato salad, sweet potato, candy, and tongue. They list the foods affected by indicating that almost any article of food which is not properly prepared and refrigerated may cause food poisoning of this type. Dack (16) noted that staphylococci grow abundantly in concentrations of salt and sugar, which kill or prevent other types from growing, such as in certain cured meats.

Investigations on growth of Staphylococcus in foods

Fitzgerald (32) in his report on frozen foods noted that they have been remarkably free from suspicion of being health hazards, both as to enterotoxic food poisoning and infectious diseases. He warned that there is a considerable possibility that pathogenic organisms may survive the freezing-storage treatment and retain sufficient viability to cause infectious diseases. There could be a source of infection in frozen foods of the very best quality because the souring would not be present to warn the user. He suggested control only through careful sanitation procedures in factories. He also mentioned that laboratory methods of detecting the incidence of toxigenic or pathogenic strains of organisms are at present inadequate and frozen foods may momentarily be implicated as health hazards.

Jones and Lockhead (49) found that of the 50 strains of staphylococci from frozen-pack vegetables, representing 980 isolations, 12 strains were positive for enterotoxin representing 411 cultures isolated. All the vegetable products tested by them were shown to harbor one or more strains capable of elaborating enterotoxin when grown in a semi-solid medium in

an atmosphere of CO_2 and air and tested with the pipette-feeding method to kittens.

Davidson and Dack (20) noted that certain staphylococci can elaborate enterotoxin in experimentally inoculated cans of corn and oysters. This was demonstrated by intravenous injection into monkeys and feeding of human volunteers. However, Staphylococcus enterotoxin was not produced in inoculated canned salmon.

Segalove and Dack (79) found that growth of staphylococci in canned foods was not necessarily correlated with enterotoxin production, and one can conclude that in the absence of growth of staphylococci no enterotoxin is produced. They reported that canned foods of low acid content (peas, corn); semi-acid content (asparagus, spinach, and string beans); medium-acid content (salmon, shrimp) were experimentally inoculated with a food poisoning strain of Staphylococcus aureus. Growth then was found to be best in low acid foods but not affected by the kind of food. In high acid foods growth did not occur. In almost all cases growth was better at 22 C (71.6 F) than at 37 C (98.6 F). The organism produced acid but no gas in the carbohydrates which it fermented; however in canned peas and corn gas was produced.

Cathcart and Merz (6) reported that natural chocolate (four ounces) and natural cocoa (three ounces) fillings made according to a given formula have a definite inhibiting action on the growth of Staphylococcus aureus. It seems, therefore, that fillings made with chocolate and cocoa,according to the specified formula without any egg,should offer little hazard from the standpoint of food illness caused by S. aureus, The pH of the fillings

and a substance or combination of substances in chocolate and cocoa are responsible for this inhibiting action. Data indicate that this substance or these substances are probably located in the non-fat part of chocolate and cocoa.

The addition of citric acid to vanilla filling in sufficient amounts to lower the pH to 3.43 to 3.65 proved effective in checking the growth of S. aureus. Lactic acid, when used in the place of citric acid, produced a better tasting cheese cake filling and effectively retarded the growth of S. aureus at pH values between 4.42 and 4.67 (8).

Smith and Iba (82) found that when pistachio nut meats were inoculated with S. aureus the organisms would increase in number up to a maximum in nine days at room temperature. The number of viable S. aureus then decreased until sterility occurred in 99 days. At 98.6 F the maximum count was reached within 48 hours after which there was rapid destruction. Viable S. aureus was not detected after 63 days.

Moorehead and Weiser (64), after a series of experiments, reported the possibility that staphylococci may be commonly carried by flies as was evidenced by the isolation of staphylococci from the digestive tract of 10 of the 50 wild flies caught and examined. The house fly, Musca domestica, may serve as a reservoir host for S. aureus, and it is possible that under suitable conditions the fly may initiate or augment a food poisoning outbreak by spreading staphylococci from infected handlers or dirty equipment to food, and from contaminated supplies to good foodstuffs which are favorable for enterotoxin production.

Enterotoxin production by staphylococci

Although Barber (1) established the relationship between the staphylococci isolated and the symptoms noted in an outbreak through human experimentation, the work was not well known until 1930 when Dack et al (13) confirmed and augmented his results.

The use of human volunteers has been largely discontinued, but since no laboratory animal seems to be as susceptible as man when fed potent filtrates, resort to human volunteers is advisable whenever inconclusive results are obtained (19).

Dolman (27) wrote that it has been accepted that the action of the enterotoxin is not directly upon the gastrointestinal tract, but rather upon some specific area of the central nervous system. The nature of the stimulus and the exact location of the susceptible area are unknown. However, Bayliss (2) presented important evidence as to the probable pathways of the reflex and the location of the center, involved in the feline vomiting reaction induced by enterotoxin.

Woolpert and Dack (99) produced symptoms similar to those in man in Rhesus monkeys with prepared toxin. They concluded that monkeys are useful in detecting the toxin in large amounts. Dack (19) fed six monkeys with veal infusion broth filtrate of the organism, and, if one vomited it was considered positive evidence of enterotoxin. When the filtrate was treated with ethanol, a large amount was required to elicit the symptoms. There was also some evidence that trypsin destroyed the enterotoxin, which was produced best in semisolid veal infusion agar in the atmosphere of 8-20 per cent CO_2.

Dolman (23) proved the evident failure of formalinization to detoxicate the enterotoxic component of certain filtrates within the time which suffices to render all the hemolytic, necrotizing, and acutely lethal substances contained therein. This fact makes it most desirable that no enterotoxigenic strain of staphylococci should be used in the production of Staphylococcus toxoid for active immunization of human beings.

Hopkins and Poland (43) concluded from their experiment that intraperitoneal injections into suckling pigs of Staphylococcus filtrates containing enterotoxin could induce vomiting, as observed in 53 of 57 pigs tested. The specificity of this response was indicated to them by failure of the animals to react when injected with autoclaved filtrates from enterotoxic strains, filtrates from nonenterotoxic strains, or filtered uninoculated medium. In contrast with other animals used for enterotoxin tests, young pigs are cheap and obtainable any time of the year. Very little special attention is required by the animal other than feeding a complete dry ration once a day and supplying it with water.

Dack (19) stated that enterotoxin is relatively heat stable in a crude form and its chemical nature difficult to determine.

The great majority of pathogenic human strains of staphylococci are definitely toxigenic under appropriate in vitro conditions of growth. Other toxigenic strains may be isolated from human staphylococcal infections. Strains of staphylococci obtained from apparently healthy mucous membranes of the nose and throat may prove extremely toxigenic when grown in vitro (24).

If a food contaminated with enterotoxigenic staphylococci be placed intermittently in the refrigerator, and the rest of the time exposed to warm temperatures, the organisms may elaborate sufficient enterotoxin to render the food poisonous. Even at the average temperature of a warm room 20-23 C,(68-73.4 F), enterotoxin production can in a short period reach a level of potency sufficient to cause poisoning in man.

Segalove and Dack (78), using a suitable strain of food poisoning Staphylococcus, found that enterotoxin could be demonstrated in a culture grown for three days at 18 C and for 12 hours at 37 C. It was not produced in cultures grown for shorter periods at these temperatures. At 9 C and 15 C (59 F) it was not produced in seven and three days, respectively. At 4 to 6.7 C enterotoxin was not formed after a period of four weeks of incubation.

The enterotoxigenicity of strains is often lost or greatly lessened by growth on artificial media. Jordon and Burrows (53) found that growth under CO_2 reactivated certain strains, while certain others,not known to produce enterotoxin,became active.

Symptoms

Dolman (27) noted that the symptomology in an acute outbreak of Staphylococcus food poisoning is so characteristic that diagnosis seldom needs be in doubt. After a symptomless interval of usually 1-4 hours followed by a period of nausea which may last from only a few minutes up to nearly an hour, sudden vomiting occurs. Several bouts of vomiting may ensue over the next few hours, and there may also be abdominal cramps

and marked diarrhea. The pulse is rapid and in severe cases may become thready, the temperature is often raised and the patient may be collapsed and apprehensive with numbness of the extremities, pallor and cold sweats. After 3-4 hours, and sometimes earlier, improvement sets in, color returns, the pulse becomes stronger, and a desire for food and drink may be expressed. Often, however, for a day or two and sometimes longer, anorexia may persist, with nausea, diarrhea or abdominal discomfort and considerable loss in weight. The mortality rate from Staphylococcus food poisoning is certainly very low, but a few apparently authentic fatalities are on record. Kodama, Hata, and Sibuya (55) reported a premature birth, with death of the infant, following an attack due to fish sausage.

Overgrowth of food poisoning staphylococci by other bacteria in foodstuffs

Counts of from 100,000 to 1 million staphylococci per gram of food material are usual, according to Dolman (27). Such low counts can only be reconciled with enterotoxic properties in a given food by supposing that a marked decline in bacterial population occurs after an early phase of rapid proliferation with maximal enterotoxin production. When a rampant Proteus strain is the competing organism, it may be impossible to detect any staphylococci in the food stuff by the ordinary method of culture on blood agar plates.

If a high dilution of the suspension is used, staphylococci may sufficiently outnumber the Proteus to grow without hindrance. In foodstuffs of long keeping qualities no staphylococcal colonies and only a few colonies of Proteus or other organisms may be isolated.

This finding may be an explanation of those salmonellosis outbreaks, in which occasional cases manifest a brief incubation period as in the report of Hinden (41), the early onset being due to preformed Staphylococcus enterotoxin.

Action of chemical agents upon enterotoxin

Haynes and Hucker (39) reported that the action of chemical agents upon enterotoxin is a subject which has for the most part been neglected. Fragmentary reports in the literature seem to indicate that the enterotoxin is a conjugate protein of the lipoprotein type. Its lipoidal nature is indicated by its ether solubility. Its insolubility in saturated solutions of ammonium sulfate, as shown by Davison and Dack (20), and its susceptibility to trypsin (62) are indications of its protein nature. Its toxicity is not altered by action of chlorine (14) and acid (pH 5.0) or rennet as shown by Minnett (62).

Effect of physical agents upon enterotoxin

The most prominent physical property of enterotoxin is its heat stability. There has been much controversy on this point. Davison and Dack (20) confirmed the instability of enterotoxin under prolonged heating, and they found that autoclaving decreased its potency.

Jones and Lockhead (49) reported that enterotoxin, besides differing from ordinary exotoxin in its resistance to heating, retained its toxicity when stored at low temperatures for two months. Although it diffuses freely into the culture medium, enterotoxin diffuses only

slightly through collodion (62). It is partially absorbed on Seitz pads during filtration but to a lesser extent than other toxic factors present in Staphylococcus filtrates. Jordon and Burrows (50) found that enterotoxin would not distill.

Antigenicity of enterotoxin

Dolman and Wilson (25, 26) and Minett (62) confirmed the demonstration of the antigenicity of enterotoxin. The first two were further able to demonstrate a flocculation reaction involving the enterotoxin and the specific antibody.

Davison and Dack (20) mixed an enterotoxic staphylococcal filtrate with an equal amount of monkey antiserum prepared against a nonfood poisoning Staphylococcus. The smallest amount of this treated filtrate that would give consistent positive results of poisoning symptoms when injected intravenously into monkeys was 0.1 to 0.2 cc. per kilo body weight.

Dack (15) found that oral administration of potent filtrates to man and rabbits was ineffective in stimulating the production in them of antibodies against the enterotoxin. Apparently some degree of immunity may be developed in individuals by oral administration of enterotoxin, but consistent results are only obtained by parenteral administration.

The control of Staphylococcus food poisoning

Husseman and Tanner (45) pointed out that every emphasis must be given to the fact that in case of Staphylococcus food poisoning the danger is not alleviated either by chilling, or in some cases, by heating

the food once it has been allowed to stand at room temperature. Neither cold nor heating to the boiling point for one half hour or more destroys the toxin once it has been formed. Once the toxin is formed there is apparently no reliable way of freeing the food of it.

They also reported that in an examination of a large number of different prepared foods, at least 20 per cent served in public places were infected with staphylococci. These foods, apparently properly handled, did not so far as was known cause illness. It is not known with certainty whether all staphylococci are potential enterotoxin formers, given the proper environments. It is more likely that only certain strains of staphylococci produce enterotoxin, but as yet there is no way of segregating these strains.

Stone (88) claimed that failure to cool milk promptly, or to maintain it and other ingredients of dairy products at a low temperature is mainly responsible for good poisoning outbreaks caused by staphylococci in milk.

Dack (17) in his discussion on bacterial food poisonings and their importance in the baking industry pointed to refrigeration as the most efficient preventive. For cream puffs, reheating after filling followed by refrigeration seems most effective.

More and more evidence accumulates to suggest that the human element, the food handlers, may be the real source of enterotoxin forming staplylococci especially in bakeries (67). Persons with colds, with suppurating lesions on hands, arms or face, or with diarrhea should be removed from food preparation and service activities. Taylor (90) advocated that food handlers be removed from their work when found by medical examination to be a possible source of contamination with Staphylococcus,

that food be not left at room temperature more than four hours, and that there be thorough cooking before serving left over food.

Jensen (47) also emphasized the importance of not keeping food for any length of time in the incubation danger zone, which he gave as from 50-120 F. The danger time within this zone is 4-8 hours depending on the extent to which the food is suitable for bacterial growth. Ham should be sliced and served at once. The same procedure should be followed with turkey or other fowls. The stuffing or dressing must not be held at temperatures above 50 or below 130 F for a longer period than four hours. Dehydrated foods do not undergo bacterial spoilage if the moisture content is below 10 per cent. They should not be held in the temperature incubation zone longer than 3 hours after the water is added before they are consumed.

Dack (17) summarized the several conditions necessary for Staphylococcus food poisoning to occur as follows:

1. Food must be contaminated with a strain of toxin forming Staphylococcus since many strains do not produce the toxin.
2. Food must contain the proper nutritive requirements including such favorable conditions as the proper amount of moisture and degree of acidity or alkalinity.
3. Suitable temperature for growth of staphylococci in food.
4. Sufficient time interval for growth and toxin formation.

He pointed out that staphylococci alone, without their growth products cause no ill effects when fed to man. At present adequate refrigeration of perishable food is the only safeguard against Staphylococcus food poisoning.

In the field of educating food handlers great strides have been made. Dodson (22), Morgan, Muse, and McKellar (65) prepared short courses for food handlers in which the necessary precautions are outlined.

With the aforementioned characteristics and growth requirements of food poisoning staphylococci in mind, it is the purpose of this investigation to find a way to inhibit them on meats by the use of a chemical agent where adequate refrigeration is not available.

Salmonella food poisoning

Importance

As Dolman (27) pointed out that food poisoning outbreaks are notorious for unreliability of statistics respecting their incidence. There is some reason to believe that *Salmonella* food poisoning may be relatively more prevalent in Britain than in North America.

The first outbreak of *Salmonella* food poisoning due to ingestion of the meat of a cow in Saxony, Germany, in 1888 was caused by *Salmonella enteritidis*. The cow had been diseased (18).

According to Hinden (41) in England and Wales 299 outbreaks of *Salmonella* food poisoning were reported from 1923-37, affecting 4418 persons of whom 102 died. In 1937 alone 45 outbreaks in the United States were reported involving 601 cases and 24 deaths. Fuchs (33) reported for the United States in 1939, 148 outbreaks of all kinds involving 3782 cases with 12 deaths, giving a relatively low incidence of *Salmonella* food poisoning. As will be shown later Salmonellae do not "poison" food, and the term *Salmonella* food infection has come to have more meaning since this infection of food poisoning type is caused by a great number of organisms. The role of *Salmonella* food infection in dehydrated foods will be discussed. The possibility that salmonellae may remain alive and virulant in dehydrated foods has added more importance to this type of outbreak.

In general, *Salmonella* cause two types of infection (18). One group apparently parasitizes only man and produces an infection and

symptoms similar to, but milder than, typhoid fever. To this group belong the species S. paratyphi and S. schottmulleri. Man may become a carrier of these organisms. The second group produces in animals a disease similar to typhoid fever in man, and if ingested in large numbers by man produces one or more of the following symptoms: nausea, vomiting, abdominal cramping, and diarrhea. Frequently fever and leukocytosis are also present. S. enteritidis is the important organism in this group. Individuals receiving a small dose of the organisms may escape without symptoms, and not all individuals react similarly.

Some outbreaks of Salmonella food infection

While the literature is full of reports, a limited number of outbreaks is mentioned here as representative of this widespread infection.

Drobinskii and Zmew (28) reported a number of cases of food poisoning by meat where the toxic effects were most probably caused by the Gaertner bacillus in Europe. Judefind (54) reported a relatively severe and protracted diarrhea presumedly due to Salmonella pullorum from the ingestion of incompletely cooked eggs. In Chile in 1947 Ortizar (69) noted an outbreak of food poisoning in 130 persons in an educational institute in Santiago. The incubation period of 12-24 hours, the sudden onset, and the symptoms of nausea, vomiting, abdominal pain, and diarrhea were noted. The attack often began with headache and chills. There were intense fever, thirst, and muscular disability. The causal organism was identified as Salmonella typhimurium.

Salmonella dublin was known to cause an outbreak of food poisoning due to contaminated milk (51).

Salmonella pullorum and S. paratyphi B (S. schottmülleri) have been isolated from hen eggs (5). Mitchel et al. (63) report that investigation of an explosive outbreak of gastroenteritis, involving a known total of at least 423 individuals, indicated that the outbreak could be traced to the ingestion of a rice custard prepared from uncooked eggs. The symptoms were severe enough to require hospitalization of 172 of the affected individuals. S. pullorum was isolated from the stools of a significant number of patients in spite of sulfonamide treatment. This organism was believed to be the cause of the epidemic and was considered to have been introduced into the rice custard in eggs obtained from infected fowl. S. pullorum is usually considered to be non-pathogenic for humans although occasional reports have indicated its isolation from stools of a few sporadic cases of gastroenteritis. In view of the common occurrence of this organism in eggs which play such an important role in human diet, the role of S. pullorum as a human pathogen must be reassessed.

Tucker et al. (91) reported that an epidemic was traced to Colby cheese from which S. typhimurium was isolated without difficulty. Experimentally it was found that the organism was viable for 362 days in cheese stored at 43-48 F. Reaud (71) described an outbreak of gastroenteritis caused by S. typhimurium. The general symptoms included vomiting, diarrhea, abdominal pain, fever, and prostration. The initial epidemiological investigation involved a consideration of the epidemic as a food-borne disease. Clinical examination of the food handlers in the institution and a cursory examination of the food consumed by the patients

failed to reveal the source of the infecting organisms. The epidemiological evidence obtained indicated that the drinking water was contaminated by rats which were believed to be the origin of the outbreak of the disease. Greenblatt et al. (37) reported a food poisoning outbreak involving 97 men over a four day period at an army camp. Commercially prepared sandwiches, all of which contained a home made mayonnaise, were apparently responsible. S. typhimurium was the etiological agent. The source of the infection was not determined.

Using 36 different bacteriophages, Lilheengen (58) has endeavoured to classify 384 strains of S. typhimurium isolated from man and different kinds of animals in several European countries, the United States, Argentina and South Africa. With a combination of 11 of the phages used or tested, 365 of the strains examined could be divided into 21 types. Strains related in epidemiological or epizootiological respects and also strains isolated from the same individual reacted to the same type of phage. Representatives of all the strains of the organism were examined after passages through mice, and the types were then found to be constant. Further information regarding the stability or uniformity of some types is still required.

McClure and Crossley (59) noted that Salmonella newport was responsible for an outbreak of acute gastroenteritis in a general hospital involving 75 cases. The incubation period was 12-18 hours. The suspected food was cold beef. Since the chef and the helper who were not ill had S. newport in their feces, they might have been carriers.

Crowe (12) described an outbreak of food poisoning due to Salmonella aertrycke from a hen's egg, which is an unusual source of the organism.

Foods affected and source of infection

As Dolman (27) has pointed out, modes of conveyance of Salmonella food infections are potentially very numerous but rather seldom revealed, owing to the incompleteness of the epidemiological investigations conducted. Savage (75) listed the chief modes of conveyance as consumption of the meat, milk, or eggs of infected animals and pollution of food by human carriers, or by infected rats and mice. From this can be seen the under-estimation of the human carrier, the fly, and the variety of animal reservoirs for Salmonella organisms disclosed in recent years.

The use of brilliant green, tetrathionate broth, bismuth sulfate agar, S. S. Agar, desoxycholate citrate agar, and Hynes medium is revealing a higher incidence of Salmonella carriers than was formerly suspected. Not only are there healthy carriers to contend with, but some types of Salmonella infection may give rise to clinical syndromes so mild as to pass unnoticed. Moreover, the convalescent carrier state may persist longer after food borne Salmonella infection than has been generally believed as pointed out by Mosher et al. (66).

The special liability of rats and mice to such infection has long been established, but it now appears that the horse, cow, sheep, pig, goat, dog, cat, turkey, duck, and chicken are subject to Salmonella infection of greater or lesser severity which manifests its presence by septicemia, abortion, gastroenteritis, or by some other ailments.

Jensen (48), in studies on 449,000 hams and 4,000 picnic shoulders, encountered no food poisoning types of microorganisms, and, therefore, food poisoning incidences from fresh pork should be very rare. However,

apparently normal hogs slaughtered for the market have been found to harbor Salmonella in their mesenteric lymph nodes up to 47.9 per cent (18). The species found are S. typhimurium, S. choleraesuis var. Kunzendorf, S. oregon, S. anatum, S. give, S. bareilly, S. derby, S. new brunswick, S. bredeney, S. enteritidis, S. lexington, S. newington, and S. worthington. This high percentage may also be due in part at least to fecal contamination.

Cherry et al. (9) found that Salmonella may be found in retail market meats. Of 250 meat samples which were examined, 13 (5.2 per cent) yielded Salmonella. Of the 170 pork products which were examined, 10 (5.9 per cent) yielded Salmonella. The incidence was found to be greater in pork products than in beef. The following types of Salmonella were isolated: S. typhimurium, S. give, S. derby, S. anatum, S. newport, S. bredeney, S. senftenberg, and S. newington. The most probable source of the Salmonella was evidently the animals from which the meats were obtained.

An examination for the presence of Salmonella in various foods purchased on the open market in Illinois (31), gave the following results: Salmonella was present in 0.6 percent of 500 shell eggs and in 3.0 per cent of powdered egg samples; in 0.2 per cent of 512 beef samples; in 17.6 per cent of 102 samples of hamburger hash; in 0.9 per cent of 327 poultry carcasses of "U. S. Inspected" grade and in 10.8 per cent of 748 uninspected birds; in 14.3 per cent of 573 "U. S. Inspected and passed" pork samples and in 26.8 per cent of 101 uninspected pork samples.

The types of Salmonella which were recorded in detail included S. typhimurium, S. oranienburg, S. bareilly, S. newport, and a number of

other species commonly associated with food poisoning. S. choleraesuis was frequently found in the pork and hamburger samples. With the exception of S. pullorum which was not uncommon in fowls, the distribution of Salmonella in human infections in the hospitals serving the areas in which the food was on sale was very similar to the prevalence in the poultry, an observation noted in several previous surveys in the United States. One of the detailed findings of interest was the frequency of S. choleraesuis in the brains, chops, and livers of hogs even in the inspected samples. There was evidence, however, that this organism is more readily killed by heat than certain other salmonellae (31).

Bornstein and Sapha (3) reported human infection due to S. saint paul, first isolated from turkeys, and to S. worthington, S. urbana, and S. manhattan, all hitherto regarded as fowl rather than human pathogens, while there have been several reports of food poisoning from duck eggs found to be infected with S. enteritidis and S. typhimurium. S. bredeney has been isolated not only from man, but has also been isolated, according to Hinshaw et al. (42) from normal swine and from a wide variety of birds, including the duck, turkey, quail, guinea fowl, and Chugar partridge.

Some investigations associated with Salmonella infection of foods

Since there are relatively fewer Salmonella food infections than Staphylococcus food poisonings, it is obvious that these organisms do not survive for a long time outside their natural hosts in most instances, except in certain adaptations as will be mentioned under the discussion. This is in sharp contrast to the common occurrence of Staphylococcus food poisoning outbreaks, which are probably due to the ubiquity of the Staphylococcus in nature.

Salmonella generally grows at temperatures ranging from 21.1-36.7 C (70-98 F), and after a two hour period at 37.2 C (99 F) reproduces every 15 minutes (48).

Lane-Claypon (56), using E. coli, S. typhosa, and S. enteritidis, has found that when a fresh broth culture is made with a small inoculum there is a period during which there is no increase in the number of bacteria present (initial stationary phase). However, when this period is over the bacteria commence to divide regularly (logarithmic growth phase); this is shown by the fact that the logarithms of the numbers plotted against time are found to fall on a straight line. This regular growth persists until (or nearly until) a maximum has been reached, after which the numbers remain more or less constant (maximum stationary phase) and then slowly decline (logarithmic death phase). This is of practical importance in that the greater the dose of Salmonella swallowed, the more acute are the symptoms and the shorter the period of onset of symptoms (48), providing of course that the strain is virulent.

An increasing amount of evidence has accumulated relating to the contamination of spray-dried whole egg powder with Salmonella; Gibbons and Moore (34), Schneider (76, 77), and Solowey et al. (83, 84). During World War II dried eggs were shipped in large quantities to Europe, and of 7,584 samples from the United States, Canada, and Argentina 754 or 9.9 per cent were found to contain salmonellae, of which 33 species were identified (18). This evidence points to the possible occurrence of Salmonella food infection from such products.

The demonstrated ability of Salmonella to multiply rapidly in reconstituted eggs at a temperature of 25-45 C (77-113 F), together

with the fact that these organisms may frequently survive scrambling temperature, point to the desirability of using egg powder immediately after its reconstitution (85).

Salmonella typhimurium and S. enteritidis, isolated from naturally infected duck eggs and inoculated into the yolk of duck eggs weighing 57-105 gm, could be recovered in culture from some of the eggs after they had been immersed in boiling water for up to 13 minutes (10).

Rettger et al. (72) found that soft boiling, coddling, and frying on one side only do not necessarily render the yolk of infected eggs free from viable S. pullorum.

Stafseth et al. (87) found that some species of salmonellae could survive in eggs stored at 25 C over a period of 12 months. They also stated that by ordinary methods of cooking: boiling, frying, poaching, and scrambling, S. pullorum frequently an inhabitant of eggs, was not always destroyed. They recommended scrambled or four-minute boiled eggs as the safest.

An investigation by Hussemann and Wallace (46) revealed that currently accepted methods of broiling or roasting markedly reduce the number of Salmonella present in chicken muscle or liver, but do not render a chicken sterile of the organism.

Properly prepared custards in normal practice will be essentially sterile in relation to Salmonella enteritidis and Staphylococcus aureus as they leave the cooking kettle or the baking oven, in the case of baked custard pies (7).

The production of "toxin" by Salmonella

Savage (74) reported "In some cases the living infective bacilli are ingested with the food with none or but little of their toxic products. In such cases the incubation period will be of appreciable duration since time must elapse before the bacilli can manufacture sufficient toxins to manifest symptoms. In other cases the bacilli may be killed during the process of manufacture or preparation for the table, and only the heat resisting toxic products will be left. These, being preformed, will exert their poisonous properties rapidly. The majority of outbreaks must be looked upon as being caused by a mixture of bacilli and toxins, the bacilli having had time to grow in the food before they were consumed."

Since this report in 1920, repeated investigations by many workers have been made to prove or disprove this theory. The reports of some of the early workers, who showed toxicity for some laboratory animals on injections with heated filtrates, can be summarized by the statement that certain heat stable soluble somatic antigens of the Salmonella group are liable to be toxic for some species of laboratory animals when injected into their blood stream. In general the various reports dating from 1920-35 indicate that suitably prepared filtrates or heat killed cultures of some strains of certain types of Salmonella may contain factors which are toxic for some species of laboratory animals. The toxic effects manifested, when such preparations are injected intravenously and those which follow oral administration, are probably due to quite different factors; and there is no evidence suggesting that either type of toxic effects is analogous to the "toxin" type of food poisoning in man.

Dack et al. (18) and Verder and Sutton (92) showed by experimentation that filtrates or heat stable products of freshly isolated cultures of Salmonella enteritidis, after passage through man, are harmless when fed to man, and that viable organisms are necessary to cause infection and illness.

Reuter (73) found that none of 35 Salmonella cultures could stand heating at 80 C for more than a few moments. Eighty minutes at 70 C or four hours at 65 C was sufficient to sterilize all the cultures. Heat stable endotoxin could not be demonstrated in any of these cultures which included six S. enteritidis strains, either in laboratory animals or when the author himself ingested boiled cultures or artificially infected boiled meat. He inferred, therefore, that meat poisoning through organisms of the Salmonella group can occur only if living organisms are present.

Although a far greater number of negative human feeding experiments, involving a wider variety of types of Salmonella and using freshly isolated strains, would need to be carried out before the categorical claim could be made that a "toxin" type of Salmonella food poisoning can not occur, the available evidence to date does not suggest that Savage's hypothesis accounts for any significant proportion of such outbreaks, according to Dolman (27).

Jensen (48) has stated conclusively that in the case of paratyphoid type of food poisoning, the paratyphoid bacillus does not form a toxin but infects the bowel directly. It was shown in the course of epidemic that only those people who ate uncooked or semicooked meat became ill, whereas those who ate cooked meat did not become ill.

Symptoms

Dack (18) stated that the symptoms of Salmonella gastrointestinal infection are characterized by nausea, vomiting, abdominal pain, and diarrhea. The onset is usually sudden. The attack may be ushered in with a headache and chill. The abdominal pain is often the first symptom and it may be griping and severe, together with persistent foul smelling diarrhea. Later in the attack the stools may be watery and sometimes of greenish color. Prostrations, muscular weakness, faintness and thirst are marked. There is almost always a rise in temperature with various nervous manifestations of restlessness, muscular twitchings and drowsiness. Oliguria may be present and herpes frequently follows.

Dolman (27) listed three main clinical forms of human Salmonella infection as follows: 1. Acute gastrointestinal irritation, 2. Typhoidal syndrome, 3. Blood stream infection. The incubation period is 12-36 hours after which there is a sudden onset of vomiting, diarrhea, and abdominal pain, accompanied by fever and prostration. The abdominal pain and diarrhea are often severe, but the illness is typically over in a few days, although sometimes of longer duration. There is an appreciable mortality rate of 1-2 per cent. In an acute outbreak the causal Salmonella is often recoverable from the feces of patients with characteristic symptoms, and may also be isolated occasionally from exposed persons who have remained symptomless or whose only complaint may be slight diarrhea.

The control of Salmonella food infection

In spite of the fact that relatively small number of cases are reported, the control of this type of infection offers considerable difficulty and is of no mean importance. One has to consider also that organisms of this type are widely found in nature, so far as natural hosts are concerned, and that typhoid fever is also caused by Salmonella typhosa.

Ostrolenk and Welch (70) showed that the house fly, Musca domestica, was responsible for spreading enteric diseases, and S. enteritidis was found to survive in the fly for the duration of its life, approximately four weeks. The organism could be found in maggots, pupae, and adult flies coming in sequence from contaminated fly eggs. In contrast to the significance of rats and mice as vectors of food poisoning organisms, the house fly appears to be much more important in food plant sanitation. Transfer of S. enteritidis infection from infected flies to mice and the retransfer of infection from infected mice to flies were also demonstrated.

Eskey et al. (29) demonstrated that the two common rat fleas, Xenopsylla cheopis and Nosopsyllus fasciatus may be infected with S. enteritidis when feeding on infected mice, and that the fleas may transmit the infection from one mouse to another by their bites. Furthermore, the feces of infected fleas also contain viable organisms in large numbers and provide an additional means by which the infection may be disseminated. Many fleas become free of the infection, but over half of

them remain infected until death. S. enteritidis infection appears to produce certain pathological conditions in the alimentary canal of fleas that tend to shorten the lives of many of them. However, some fleas survived the infection for more than two months.

Meat Inspection laws in Canada and the United States seem to have done much in preventing the spread of Salmonella. Also refrigerating units have played an important part. This seems evident when the number of outbreaks in Europe is compared with those in the above two countries. However, as mentioned, statistics regarding such incidences may not represent actual cases, because many unidentified cases may pass for common gastrointestinal upsets of obscure etiology. The control of rodents is also an important phase of the work in preventing food infection of this type, and the house fly must be eradicated. Milk and cheese may become contaminated from a diseased cow or subsequent handling. Pasteurization of milk is effective in destroying Salmonella. It must be remembered that in this type of Salmonella infection the organisms are pathogenic for animals, producing in animals a disease similar to typhoid fever in man, and are different only from the pathogenic forms attacking man.

Since there are many ways and means, by which meats can be contaminated on the surface with Salmonella, the control of such incidence by the use of a chemical agent is the goal of this investigation. Salmonella infection is often associated with "meat poisoning", and a chemical which will inhibit food poisoning organisms on meat should be valuable, because not only can it be used to treat meat sold without

refrigeration but it can also be used to treat market meat before it is refrigerated. Refrigeration, as afore mentioned, is not an effective way of destroying food poisoning organisms.

For very practical procedures of detection and isolation of food poisoning organisms from dairy products, a concise article by Newman (67) is recommended.

Effects of some common chemicals on food poisoning organisms

An investigation by Voegeli (93) revealed that cutting utensils are responsible to a great extent for bacterial contamination of pre-packaged fresh meat. Mallmann and Churchill (61), in their article on the control of microorganisms in food storage rooms, stated "If we are to control the development of microorganisms on the surface of beef during short time storage, then either ultraviolet light or carbon dioxide atmosphere may answer the purpose effectively. If microorganisms are contaminating food produce through contacts with utensils, table surfaces, etc. then a chemical sanitizer is indicated. If contamination on wall and floor surfaces is a source of trouble, then a chemical agent, preferably with residual activity, should be selected." "Because the quarternary compounds exhibit considerable bacteriostatic action on spores, it would seem that these compounds would be preferable for use following good cleaning in preference to the hypochlorites."

Hucker and Haynes (44) found that, in veal broth, acetic acid in concentration as low as 0.15 per cent can bring about a decided reduction in the number of food poisoning staphylococci in 48 hours, and at the end of seven days media containing 0.2-0.3 per cent of the acid were practically free of organisms. Hence acetic acid has an inhibiting effect in low concentration and a definite killing effect in higher concentrations.

In studies on food poisoning staphylococci by Nunheimer and Fabian (68), acetic acid has been found to possess a marked germicidal power in comparison to some commonly occurring organic acids in foods as well as hydrochloric acid. The order is acetic, citric, lactic, malic, tartaric and hydrochloric acids. The decreasing order of antiseptic action was found to be acetic, lactic, citric, malic, tartaric and hydrochloric acids. In this study stress was placed upon food poisoning staphylococci.

Levine and Fellers (57), in their study of the effect of acetic acid on microorganisms related to food spoilage, found that acetic acid possesses toxicity for bacteria in excess of that which can be attributed to pH alone. The inhibiting and lethal action of acetic acid is very pronounced.

INHIBITING AND LETHAL CONCENTRATIONS OF ACETIC ACID
(After Levine and Fellers)

Organism	Inhibiting pH	Inhibiting Acidity	Lethal pH	Lethal Acidity
		Per cent		Per cent
Salmonella aertrycke	4.9	0.04	4.5	0.09
Staphylococcus aureus	5.0	0.03	4.9	0.04
Phytomonas phaseoli	5,2	0.02	5.2	0.02
Bacillus cereus	4.9	0.04	4.9	0.04
Bacillus mesentericus	4.9	0.04	4.9	0.04
Saccharomyces cerevisae	3.9	0.59	3.9	0.59
Aspergillus niger	4.1	0.27	3.9	0.59

Dehydroacetic acid (3-acetyl-6-methyl-1,2-pyran-2,4(3)dione) and its sodium salt have been used as mycostatic agents on bread, fruits, vegetables, and dairy products. The compounds are relatively nontoxic

(81,86,98) at concentrations suitable for use, and at which they are also odorless, colorless, and tasteless. However, there is some decrease in antimicrobial activity of the acid and its sodium salt with an increase in pH (97), and this is probably one of the reasons why their use to control food poisoning organisms on meat in this investigation was not satisfactory. They are less active in an alkaline range than in an acid range.

INHIBITING CONCENTRATIONS OF DEHYDROACETIC ACID
(After Wolf)

Test Organism	Concentration Permitting Growth (Per cent)	Concentration That Inhibits Growth (Per cent)
Bacillus subtilis	0.2	0.3
Escherichia coli	0.3	0.4
Salmonella pullorum	0.2	0.3
Salmonella typhosa	0.1	0.2
Staphylococcus aureus	0.2	0.3
Streptococcus pyogenes	0.2	0.3

Fabian and Bloom (30) found dichloracetic acid to be less and trichloracetic acid still less effective than monochloracetic acid in preserving action. They also found benzoic acid to inhibit the growth of microorganisms to about the same extent as monochloracetic acid under practical conditions, and both chemicals to be more effective against yeasts than acid producing bacteria. The use of monochloracetic acid is also prohibited by the Food and Drug Administration, and its utility in the United States is therefore not possible.

McLean et al. (60) found that chloramphenicol (chloromycetin) can inhibit many Salmonella species in vitro at 2.5 ug./ml., and resistant strains at 5.0 ug./ml. in the culture medium. It can inhibit Staphylococcus in the concentration of 2.5-10.0 ug./ml.

Chloramphenicol has been used clinically in the treatment of fevers of the enteric group with success, and several favorable reports have been published in various medical journals. Reviews in many numbers of Bulletin Of Hygiene reveal its appreciation in other countries.

Chloramphenicol (chloromycetin), aureomycin, and terramycin were added respectively to freshly ground beef in concentrations ranging from 0.5 to 2.0 ppm and their effect noted on the total bacterial count as well as the keeping quality of the meat. Ground beef containing any of the above antibiotics spoiled after approximately 10 days, while the controls spoiled after 5 days. Ninety per cent of the beef microflora studied showed marked susceptibility to the above antibiotics (35).

PART I. GROWTH OF FOOD POISONING ORGANISMS ON BEEF AND PORK

Introduction

It is generally believed that cooked meat is more favorable to bacterial growth than fresh meat, probably due to the breaking down of nutrients to more assimilable forms. It is interesting, therefore, to determine the differences in growth of food poisoning staphylococci and salmonellae on raw beef and pork, specially on raw lean meat taken from the depth of retail cuts, on cooked lean meat, and on the surface of raw lean meat prepared and handled in retail trade.

Since meat may be contaminated on the surface with food poisoning staphylococci and salmonellae from the time of killing to storage and sale, this is a study of the viability of the organisms on meat kept at different atmospheric temperature ranges exclusive of a very high range such as 32.2-37.8 C (90-100 F) or a low range such as freezing.

The present study was conducted to determine the optimum temperature range between the very high and low temperatures so that the meat could be sanitized with the chemical agents being studied to determine whether such agents would be effective at this optimum temperature range.

Brewer (4), in his work with the bacterial content of market meats, concluded that prepared meats usually contained more bacteria than fresh meats, and that of the bacteria encountered in the different meats the colon group predominated.

Jensen (48) mentioned that coliform organisms are abundant and ubiquitous on the killing floor, and almost invariably found on the surfaces of the carcasses which are exposed during killing floor operations.

Voegeli's work (93) showed that the cutting utensils were responsible to a great extent in bacterial contamination of prepackaged fresh meats.

One can associate the facts, therefore, that coliform organisms found on the surface of carcasses can contaminate the cutting utensils, which subsequently spread the organisms to all cuts of market meats as found by Brewer (4). In addition there is the possibility of other sources of contamination with these organisms such as the hands of the handlers.

Waksman (94) extensively reviewed the antagonistic relation between microorganisms living in association including the two-sided antagonism existing between Escherichia coli and certain salmonellae. However, strains of E. coli varied in their inhibitive power against salmonellae while species of salmonellae varied in their resistance to inhibition of E. coli.

Two pure cultures of E. coli were isolated. One from a beef carcass and the other from a human stool. Both inhibited Salmonella choleraesuis var. kunzendorf and Salmonella enteritidis when grown in association with the salmonellae (36).

Test organisms

Three strains of food poisoning Staphylococcus, numbered 172, 178, and 196 were used in this investigation. The original cultures had been obtained from Dr. G. M. Dack, Food Research Institute, University of Chicago. The organisms had been previously isolated from chipped beef and two different samples of ham, and their toxicity had been proved several times by the use of monkeys.

Eight species of test salmonellae were obtained from the stock cultures of Michigan State College. The organisms had been observed to possess the usual characteristics of the species. The organisms were Salmonella choleraesuis (var. Kunzendorf), Salmonella paratyphi, Salmonella enteritidis, Salmonella gallinarum, Salmonella schottmülleri, Salmonella typhimurium, Salmonella pullorum, and Salmonella typhosa.

Salmonellae are known to have caused food poisoning or rather food infection, as it might be called according to pathogenesis, of distinct nature. Bergy's Manual of Determinative Bacteriology, 6th edition, states that only five serotypes of salmonellae are of special interest in the field of human medicine, namely S. paratyphi A, S. paratyphi B, S. paratyphi C, and S. sendai. While these organisms are human pathogens, the literature is full of descriptions of food poisoning cases caused by salmonellae of animal origin, and more attention is being given to them at present. Sanitation authorities, for example, are mindful of the control of rodents as an important phase in preventing food poisoning from S. typhimurium.

Media used

Due to the large numbers of bacteria present on pieces and cuts of meats resulting from handling and coming in contact with various objects, it becomes necessary, in working with fresh meats, to employ media of high selectivity in order that differential counts of the food poisoning staphylococci and salmonellae on them can be made. Market meats, of course, contain appreciable numbers of organisms (93). A series of trials proved that Difco Chapman-Stone medium is very suitable for differential counting of staphylococci, especially the usual food poisoning type, although others often produce the same Stone reaction, a clear halo around the colonies. The surface colonies are distinct and separate when plating is properly carried out.

Since the Salmonella species used were found to grow on Difco Salmonella Shigella Agar (S. S. Agar), this medium was very satisfactory for differential counts after they had grown on the meat for a period of time. The medium exerts delaying and inhibitory effects on other bacteria usually present on the meat, and after 12-14 hours of incubation at 37 C salmonellae appear in grayish or pinkish gray colonies if bacterial colonies on the plate are not crowded. Lactose-fermenters appear in brick red colonies.

Experimental

Food poisoning staphylococci and salmonellae varied markedly in growth when grown on pieces of raw beef and pork taken from different

retail cuts probably due to varied moisture content and amount of nutritive materials on the inoculated surface. In order to obtain comparative counts in plating blocks of lean beef and pork, about 5 grams were taken from the same retail cuts and prepared as follows:

(a) Lean pieces of raw meat cut from 1 cm under the surface aseptically were designated as "deep lean meat".

(b) Lean pieces of raw meat cut from the surface of retail cuts, using sterile equipments to avoid further addition of bacteria, were designated as "autoclaved lean meat".

(c) Lean pieces of meat cut 1 cm beneath the surface and autoclaved at 15 pound pressure for 15 minutes to destroy all spores present were designated as "autoclaved lean meat". To conserve moisture during autoclaving each piece of meat was placed at the edge of the bottom of a closed and slanted Petri dish; the piece of meat rested on the lowest angle formed by the wall and the bottom of the Petri dish.

The deep lean meat (a), the surface lean meat (b), and the autoclaved lean meat (c) were inoculated with 24 hour nutrient broth cultures of the food poisoning organisms and left covered in Petri dishes at 21.1-26.7 C (70-80 F) for 24 hours before plating in order to compare the amount of growth on them.

To compare the influence of temperature, other pieces of deep lean meat, taken at the same time from the same chunk of meat as the above deep lean meat pieces, were also placed in Petri dishes and inoculated with a loopful of the respective food poisoning organisms. They

were incubated for 24 hours at different temperature ranges before plating. The approximate number of the different organisms in a loopful were determined by plating and are presented in, tables 3 and 4 in the first column designated "inoculum". These numbers were also used as the starting points in plotting the graphs in figures 3, 4, 5, and 6. Uninoculated pieces of meat were used as controls and were incubated at the different temperature ranges for 24 hours before plating for staphylococci and salmonellae. Control pieces of fresh deep lean meat were also immediately plated to determine total numbers of bacteria by using tryptone-glucose-yeast-extract agar.

Temperature ranges used for incubating the different pieces of meat were 4.4-10.0 C (40-50 F), 10.0-15.6 C (50-60 F), 15.6-21.1 C (60-70 F), 21.1-26.7 C (70-80 F), and 26.7-32.2 C (80-90 F). In each case moisture was supplied by a basin of water placed directly below the closed Petri dish containing the inoculated meat so that excessive dessication would not take place.

As will be shown later, the results of the experiments indicated that food poisoning organisms on lean beef and pork did not increase in numbers at 4.4-10.0 C (40-50 F) during 24 hours but increased markedly at 21.1-26.7 C (70-80 F) and 26.7-32.2 C (80-90 F). An experiment to determine the growth of food poisoning organisms on pork rind and the external fat of the beef carcass was also carried out by using the above temperature ranges for incubation. Blocks of about 5 grams of lean pork with the rind and beef with the external fat were cut from the surface. Each of the 24 hour broth cultures of the organisms used was inoculated

on the pork rind and the beef fat. After the inoculation the inoculated materials were incubated for 24 hours at the above three temperature ranges before plating.

The inoculation in all cases was done by touching a spot in the center of the surface of the meat to be inoculated with a 4 mm wire loop containing the broth culture of the organism until the contents ran down onto the spot. This was done to obtain similar, inoculated areas.

In inoculating a loopful of broth culture on the surface of pork and/or beef as described above there was some doubt as to whether the nutrient broth might increase the nutrients thereon resulting in more growth than there would be without the broth. To determine the effect of the broth on the growth of the organisms, 24 hour broth cultures of the organisms were centrifuged and washed three times with physiological saline. The final suspensions were allowed to settle for 90 minutes until they appeared similar in concentration to 24 hour broth cultures which had not been shaken; the suspensions were then used for inoculation. The results of plating from lean pork and beef, inoculated with such washed bacteria and incubated at 26.7-32.2 C (80-90 F), were essentially the same as when regular 24 hour broth cultures were used for inoculation (last columns of tables 3 and 4). It was, therefore, concluded that the broth had little or no effect on the growth of the bacteria.

The usual pour plate method of plating was found unsatisfactory when using Chapman-Stone medium or S. S. agar, especially in

differentiating numerous species of bacteria on meat in the S. S. agar. The Chapman-Stone medium was not designed for making pour plates because after rehydration it had a heavy consistency and solidified at a temperature well above 45 C. S. S. agar did not show marked differences between colonies of salmonellae and other organisms found on beef and pork, if grown under the surface; it was quite impossible to differentiate these colonies after 12-16 hour incubation and after longer periods of incubation, 16-24 hours, the medium was not inhibitory and allowed growth of numerous organisms.

However, surface colonies of salmonellae on S. S. agar were characteristic and grew much larger than colonies of other organisms after 14-21 hour incubation at 37 C. Colonies of Staphylococcus on the surface of Chapman-Stone medium also grew larger and were more uniform in size than subsurface colonies.

A surface plating method to assure differentiation, separation, and uniformity in size of colonies of staphylococci and salmonellae was developed for more accurate and easy counting. The method is as follows:

Chapman-Stone medium and S. S. agar were prepared as usual and poured into Petri dishes in 25-30 ml quantities. It was necessary to pour a larger volume than usual in the Petri dish because after solidification the plate was to be dried at 37 C for 24 hours and at room temperature for another 48 hours before use.

One-tenth ml. of the properly diluted material to be plated was delivered from a 0.1 ml serological pipette on the surface of a prepared agar plate. This inoculum was quickly spread over the surface by means

of a spreading wire. The spreading wire was made by bending an ordinary inoculating needle at a 65 degree angle 170 mm from the handle and bending again at a 70 degree angle 23 mm from the first bend and 7.5 mm from the tip; it was a wire bent twice to have a 23 mm length used for spreading between the first bend and the second bend. The tip was, therefore, upturned to prevent digging into the agar while spreading. The wire was found to give more uniform results than the usual glass rod used for spreading probably because constant amounts of inoculum adhered to the wire due to its small diameter. Furthermore, wires were easy to duplicate in these dimensions and could be conveniently sterilized in the flame.

When the plate containing the medium was properly dried the spreading could be done rapidly by rotating the Petri dish in the left hand while the right hand moved the spreading wire to and fro. The moisture of the inoculum was absorbed in a few seconds which prevented the development of spreading colonies. All results could be read the next day after plating (Fig. C-1, C-2, C-1a, and C-2a).

The swabing method to remove bacteria from the meat (61) after repeated trials showed wide variations in results. This fact might be due to varied moisture content and adhesion of the bacteria on the surface of the pork and beef even on different parts of the same cuts. The surface of pork and beef also was irregular.

The inoculated and incubated pieces of lean pork and beef and/or their coverings as well as the control pieces were dropped into dilution bottles containing 10 to 100 ml of distilled water. The bottles were shaken 50 times before suitable dilutions were made from them and 0.1 ml quantities were immediately plated in duplicate from each dilution.

Fig. C-1. Plates made from the same dilution of staphylococci found on raw pork using Chapman-Stone medium after 18 hour incubation. Left - colonies on surface spread-plate. Right - regular pour plate showing needle point subsurface colonies.

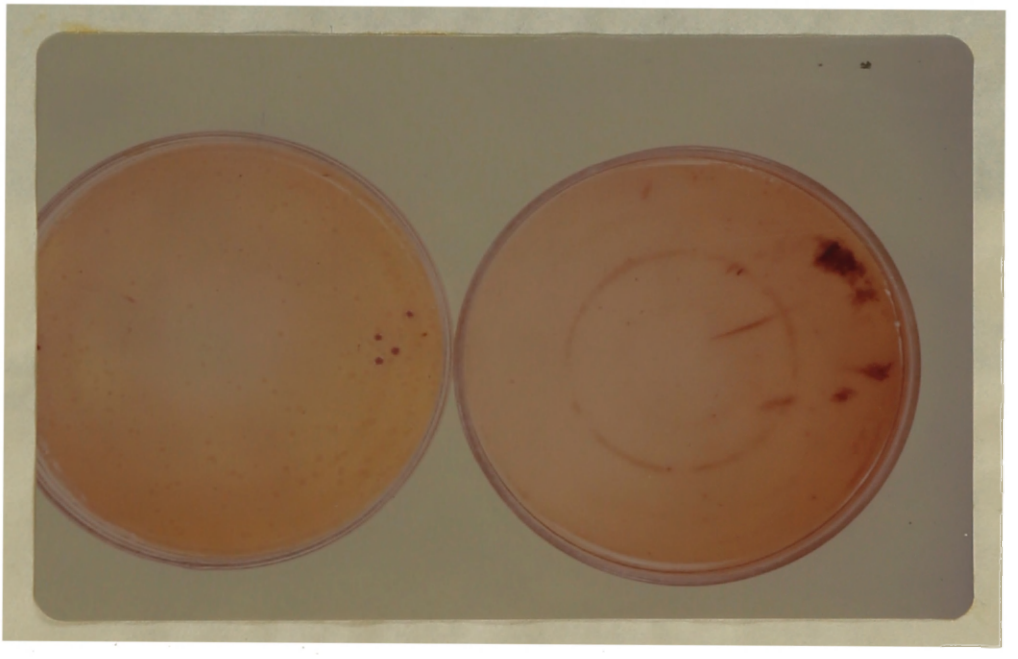

Fig. C-2. Plates made from the same dilution of Salmonella typhimurium recovered from raw pork using S. S. agar after 16 hour incubation. Left - surface spread-plate showing red colonies- coliforms; yellowish gray colonies - Salmonella. Right - regular pour plate showing pin point subsurface colonies; dark areas - sulfite producing colonies.

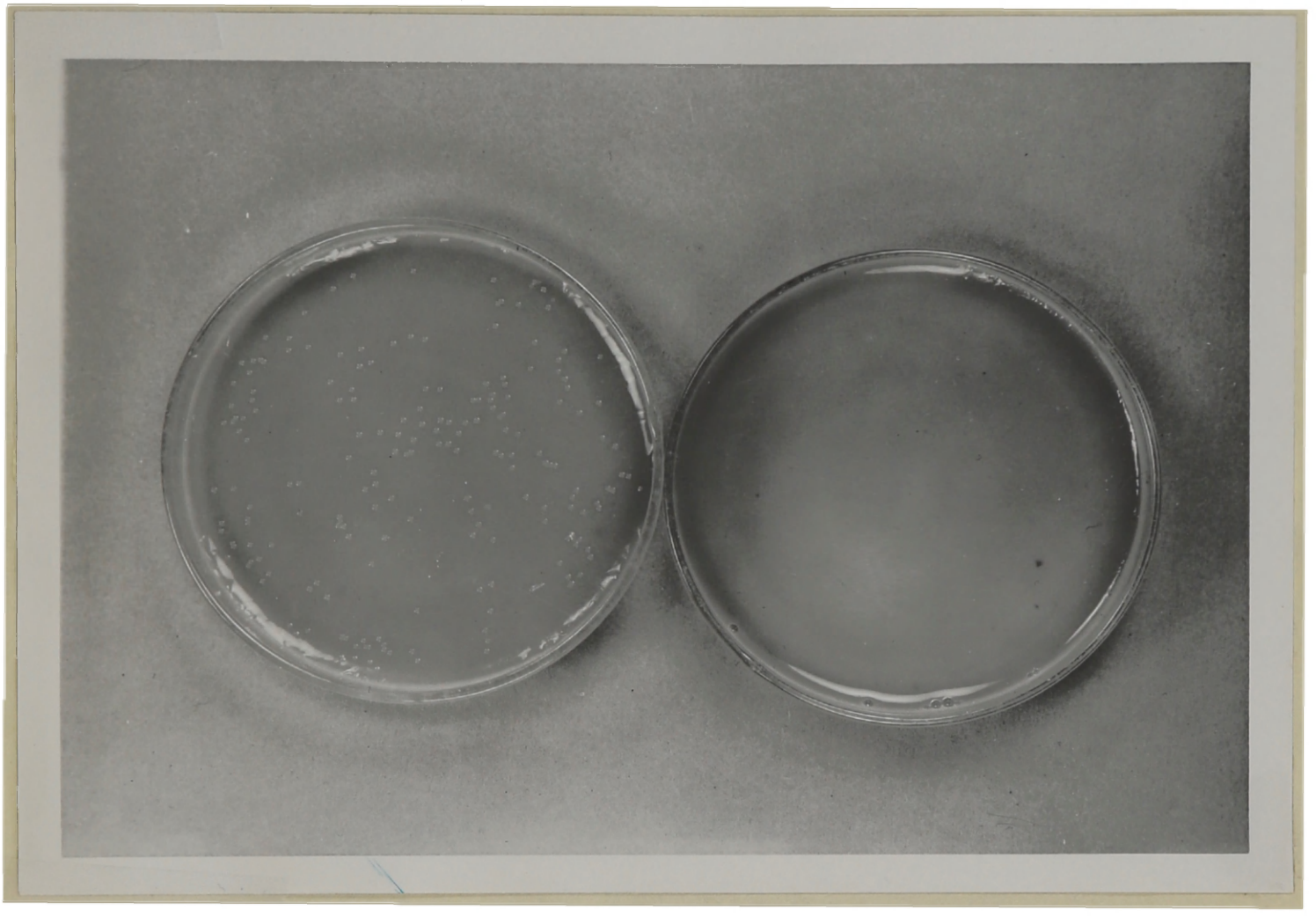

Fig. C-1a. Black and white supplement to Fig. C-1 showing colonies on surface of spread-plate, left and minute subsurface colonies on poured plate, right.

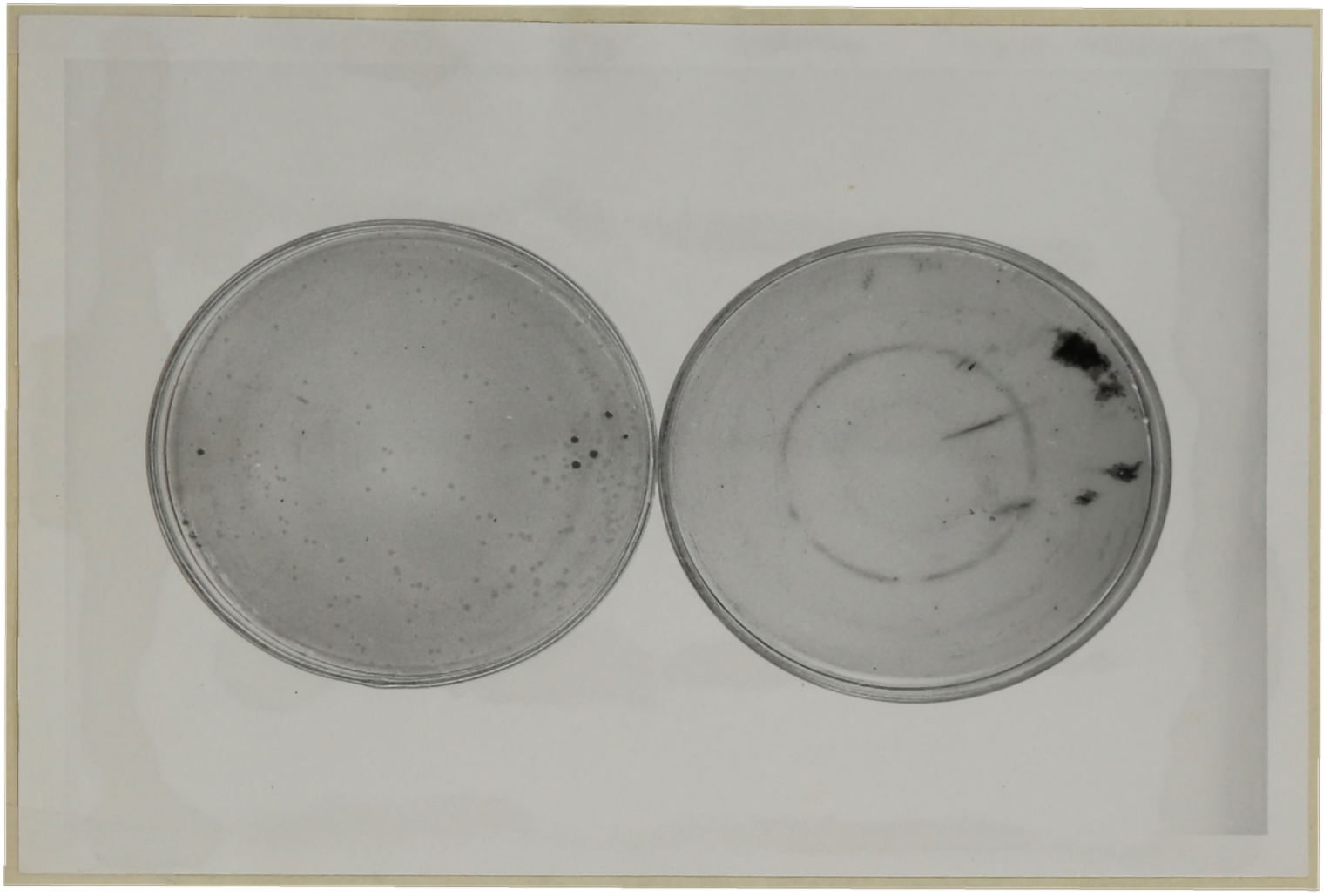

Fig. C-2a. Black and white supplement to Fig. C-2. Left plate shows surface spread-plate with large distinct coliform and Salmonella colonies. The right plate shows minute subsurface colonies in the poured plate.

Results and Discussion

As reviewed under the topic of "general consideration" (page 5), pork and beef were found to have rather large numbers of bacteria on the surface (89,38). However, most of the early work done on the bacteriology of tissues of normal animals indicated that muscle tissues were sterile. Hauser, Neisser, Opitz, Messner, Amako, Wyssokowitsch, Thole, Hass, and Kuster as cited by Jensen (48) believed they were sterile. Recently Goldberg *et al.* (35) studied the inherent microflora of beef and differentiated 94 organisms representing 12 genera isolated from the deep tissues.

While numbers of bacteria on the surface of pork and beef may be large depending on the contamination from cutting utensils as one of the contributing factors (93), the control plating from fresh lean pork and beef, taken 1 cm beneath the surface, showed comparatively very small counts ranging from 3 to 60 per sq cm. It was evident that the cutting utensils, even when sterile, might still convey some surface bacteria to the deep lean meat.

The data persented here show no marked differences when poisoning staphylococci were grown at 21.1-26.7 C (70-80 F) on deep lean, autoclaved lean, and surface lean pork and beef. Likewise, salmonellae did not vary significantly in numbers when grown on fresh deep lean meat or autoclaved deep lean meat but showed a great reduction when grown on surface lean pork and beef (tables and figures 1 and 2). The reduction in the bacterial counts on surface lean meat may be attributed

to the antagonism offered by other organisms growing in association with them, and to less available moisture because some doubtless had evaporated.

The control pieces of deep lean pork and beef placed in various temperature ranges were by no means sterile, (tables 3 and 4). However, the initial contamination of these pieces might nog have been great enough to render the growth of salmonellae significantly different from that on autoclaved lean beef and pork.

Food poisoning organisms appeared to grow better on pork than on beef especially at 21.1-26.7 C (70-80 F) and also at 26.7-32.2 C (80-90 F). No marked increase in numbers of food poisoning staphylococci was observed on lean beef at 10.0-15.6 C (50-60 F) during 24 hours, but the organisms grew on lean pork stored at this temperature range, (tables and figures 3 and 4).

At 10.0-15.6 C (50-60 F) 2 of the 8 species of salmonellae used increased in numbers on lean pork while 5 species decreased in numbers and 1 remained constant. On lean beef at this temperature range 2 of the 8 species of the salmonellae increased in numbers while 6 species showed decreases, (tables 3 and 4). This fact indicated that salmonellae were not so active in growth and reproduction at this temperature range with beef and pork as the media.

Temperature ranges between 15.6-32.2 C (60-90 F) were favorable to all test organisms, the optimal range being 26.7-32.2 C (80-90 F). No increase of the test organisms was observed at 4.4-10.0 (40-50 F) on lean pork and beef, (tables and figures 3 and 4).

External fat of the beef carcass appeared to be rather more favorable to growth of food poisoning staphylococci than pork rind. Salmonellae, however, grew better on the pork rind than on the external fat of the beef carcass, (tables and figures 5 and 6). These coverings compared with the lean meat, were poor media and allowed but little growth of the organisms used, (tables 3, 4, 5 and 6). These results indicated that the nutrients present on pork rind and external fat of the beef were limited or possibly not so readily available to the food poisoning bacteria. Therefore, pork and beef should be kept and handled with such natural coverings on whenever possible.

At 26.7-32.2 C (80-90 F) there was generally less growth of food poisoning organisms on pork rind and external fat on the beef than at 21.1-26.7 C (70-80 F), (tables and figures 5 and 6). This was apparently due to the evaporation of the surface moisture. These coverings, of course, contained much less moisture than lean meat.

Summary

1. There was no significant differences in the number of food poisoning staphylococci growing on raw fresh and cooked beef and pork.
2. Salmonellae did not differ significantly in their growth on the raw fresh and cooked lean beef and pork taken from a 1 cm depth of retail cuts. They grew much less on the surface of retail cuts of raw beef and pork probably due to the antagonism offered by other organisms grown in association with them and also due to desiccation.

3. Pork is a better medium for growth of food poisoning staphylococci and salmonellae than beef.
4. The atmospheric temperature range, exclusive of a very high range such as 32.2-37.8 C (90-100 F) or a low range such as freezing, optimal for growth of food poisoning staphylococci and salmonellae on lean beef and pork is 26.7-32.2 C (80-90 F). No growth was observed in 24 hours at 4.4-10.0 C (40-50 F) which is the usual refrigerating zone of household refrigerators.
5. Pork rind is a better medium of growth for food poisoning staphylococci and salmonellae than the external fat of the beef carcass. However, both the rind and the fat are poor media compared to the lean meat. Pork and beef, therefore, should be kept and handled with these natural coverings on whenever possible.

Table 1

Growth of food poisoning organisms on beef at 23.9 ± 2.8 C for 24 hours. Count in millions

Organism	Inoculum	Deep lean beef	Autoclaved lean beef	Surface lean beef
Staphylococcus 172	2.000	2,500.000	3,700.000	4,500.000
Staphylococcus 178	1.400	1,800.000	1,900.000	2,500.000
Staphylococcus 196	1.900	2,100.000	2,000.000	2,000.000
Salmonella choleraesuis var. kunzendorf	1.700	260.000	240.000	230.000
Salmonella paratyphi	.060	2.200	3.000	.100
Salmonella enteritidis	.690	1.200	2.900	.800
Salmonella gallinarum	.540	.420	.300	.000
Salmonella schottmuelleri	.050	22.000	20.000	.500
Salmonella typhimurium	1.000	95.000	110.000	42.000
Salmonella pullorum	.230	5.000	4.300	.400
Salmonella typhosa	.006	25.000	19.000	1.200

Table 2

Growth of food poisoning organisms on pork at 23.9±2.8 C for 24 hours. Count in millions

Organism	Inoculum	Deep lean pork	Autoclaved lean pork	Surface lean pork
Staphylococcus 172	2.000	7,800,000	6,800.000	4,200.000
Staphylococcus 178	1.400	5,600.000	4,300.000	4,300.000
Staphylococcus 196	1.900	4,200.000	3,800.000	3,500.000
Salmonella choleraesuis var. kunzendorf	1.700	1,400.000	1,700.000	1,200.000
Salmonella paratyphi	.060	4.500	5.000	2.900
Salmonella enteritidis	.690	450.000	460.000	50.000
Salmonella gallinarum	.540	150.000	200.000	20.000
Salmonella schottmuelleri	.050	240.000	210.000	80.000
Salmonella typhimurium	1.000	1,900.000	1,700.000	70.000
Salmonella pullorum	.230	670.000	530.000	100.000
Salmonella typhosa	.006	9.000	6.000	.020

Table 3

Growth of food poisoning organisms on beef at various temperature ranges.
Count in millions.

Organism	Inoculum	7.2±2.8 C	12.8±2.8 C	18.3±2.8 C	23.9±2.8 C	29.4±2.8 C
Staphylococcus 172	2.000	.600	1.500	22.000	2,500.000	5,300.000
Staphylococcus 178	1.400	.750	1.200	6.500	1,800.000	5,400.000
Staphylococcus 196	1.900	1.000	1.100	9.500	2,100.000	9,600.000
Salmonella choleraesuis var. kunzendorf	1.700	1.300	1.500	6.100	260.000	630.000
Salmonella paratyphi	.060	.040	.010	.470	2.200	4.400
Salmonella enteritidis	.690	.140	.130	.400	1.200	16.000
Salmonella gallinarum	.540	.040	.050	.090	.420	4.000
Salmonella schottmuelleri	.050	.040	.075	.550	22.000	170.000
Salmonella typhimurium	1.000	1.000	.900	7.800	95.000	100.000
Salmonella pullorum	.230	.100	.100	.110	5.000	100.000
Salmonella typhosa	.006	.005	.110	.190	25.000	190.000
Control (Staphylococcus on meat)	natural	.002	.001	.010	1.200	6.200
Control (Salmonella on meat)	natural	0	0	0	0	0

Table 4

Growth of food poisoning organisms on pork at various temperature ranges.
Count in millions.

Organism	Inoculum	7.2±2.8 C	12.8±2.8 C	18.3±2.8 C	23.9±2.8 C	29.4±2.8 C
Staphylococcus 172	2.000	.800	3.600	110.000	7,800.000	18,000.000
Staphylococcus 178	1.400	.800	2.300	30.000	5,600.000	6,200.000
Staphylococcus 196	1.900	.950	2.300	35.000	4,200.000	5,400.000
Salmonella choleraesuis var. kunzendorf	1.700	1.400	5.400	120.000	1,400.000	3,300.000
Salmonella paratyphi	.060	13.000	.062	.950	4.500	82,000.000
Salmonella enteritidis	.690	.520	.270	1.600	450.000	1,900.000
Salmonella gallinarum	.540	.480	.091	4.800	150.000	940.000
Salmonella schottmuelleri	.050	.034	.050	1.600	240.000	430.000
Salmonella typhimurium	1.000	.800	.065	62.000	1,900.000	4,900.000
Salmonella pullorum	.230	.180	.100	.400	670.000	3,600.000
Salmonella typhosa	.006	.004	.002	.100	9.000	80.000
Control (Staphylococcus on meat)	natural	.002	.001	.050	120.000	420.000
Control (Salmonella on meat)	natural	0	0	0	0	0

Table 5

Growth of food poisoning organisms on external fat of the beef carcass at various temperature ranges. Count in millions.

Organism	Inoculum	7.2±2.8 C	23.9±2.8 C	29.4±2.8 C
Staphylococcus 172	2.000	2.300	63.000	21.000
Staphylococcus 178	1.400	1.900	83.000	62.000
Staphylococcus 196	1.900	.980	20.000	59.000
Salmonella choleraesuis var. *kunzendorf*	1.700	.0004	22.000	19.000
Salmonella paratyphi	.060	.0003	.020	.006
Salmonella enteritidis	.690	.0004	.730	.260
Salmonella gallinarum	.540	.0009	.280	.250
Salmonella schottmuëlleri	.050	.0006	.970	.080
Salmonella typhimurium	1.000	.0002	.320	.670
Salmonella pullorum	.230	.082	.011	.004
Salmonella typhosa	.006	.0003	.200	.070
Control (*Staphylococcus* present)	natural	.210	8.500	13.000
Control (*Salmonella* present)	natural	0	0	0

Table 6

Growth of food poisoning organisms on pork rind at various temperature ranges. Count in millions.

Organism	Inoculum	7.2±2.8 C	23.9±2.8 C	29.4±2.8 C
Staphylococcus 172	2.000	2.200	14.000	20.000
Staphylococcus 178	1.400	1.900	29.000	18.000
Staphylococcus 196	1.900	3.300	51.000	40.000
Salmonella choleraesuis var. *kunzendorf*	1.700	.0005	1.800	.006
Salmonella paratyphi	.060	.0003	.060	.001
Salmonella enteritidis	.690	.0004	15.000	10.000
Salmonella gallinarum	.540	.001	18.000	20.000
Salmonella schottmuelleri	.050	.001	2.000	2.300
Salmonella typhimurium	1.000	.014	17.000	16.000
Salmonella pullorum	.230	.0007	.015	.002
Salmonella typhosa	.006	.0012	.080	.120
Control (*Staphylococcus* present)	natural	.640	11.000	14.000
Control (*Salmonella* present)	natural	0	0	0

Keys to the Opposite Figure

Number	Line	Species of Organisms
1.	———	Staphylococcus 172
2.	——·——	Staphylococcus 178
3.	——·——	Staphylococcus 196
4.	———	Salmonella choleraesuis var. kunzendorf
5.	— — —	Salmonella paratyphi
6.	—·—·—	Salmonella enteritidis
7.	.....	Salmonella gallinarum
8.	—··—··—	Salmonella schottmuelleri
9.	— - — - —	Salmonella typhimurium
10.	—+—+—+—+—	Salmonella pullorum
11.	—#—#—#—#—	Salmonella typhosa

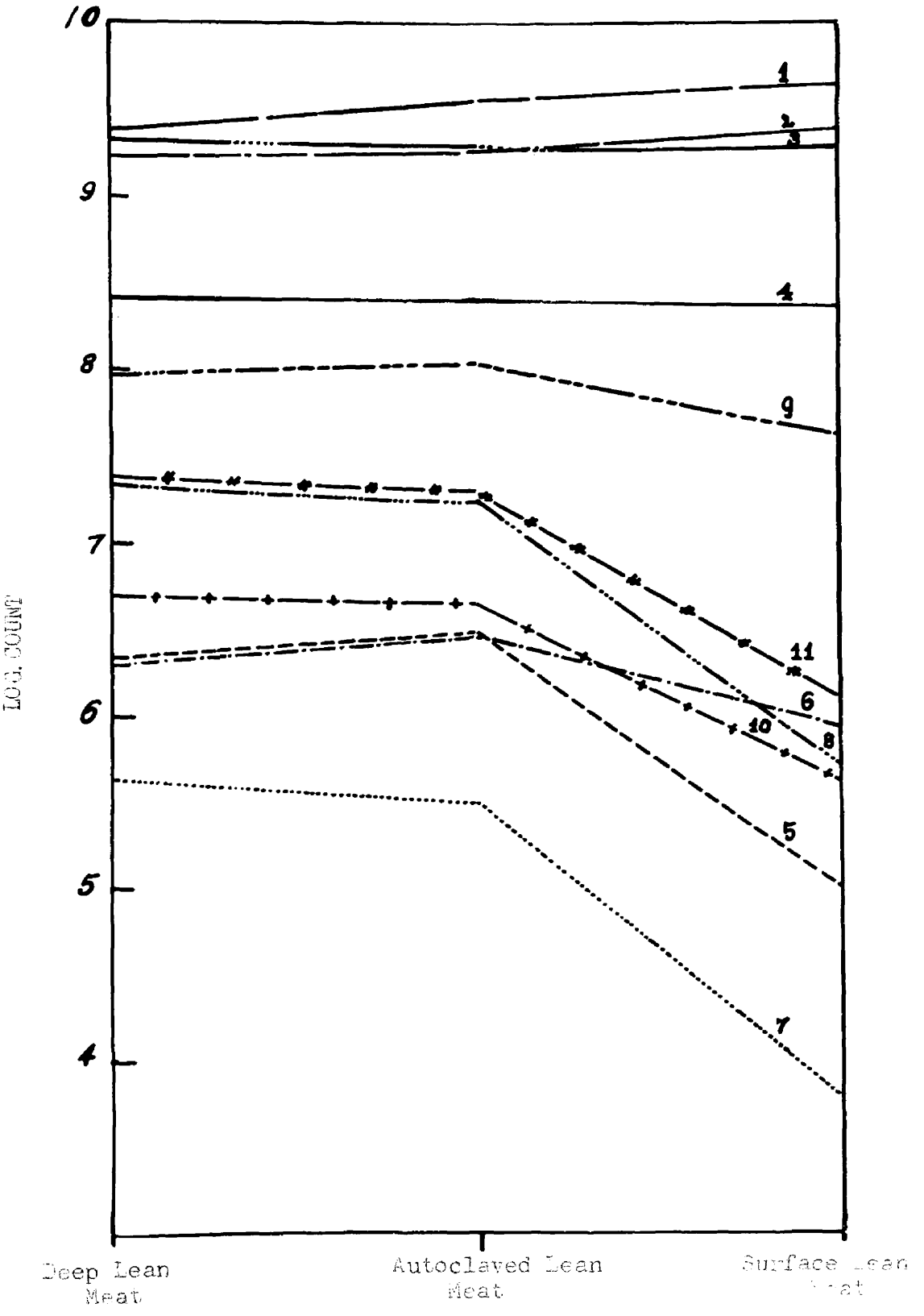

Fig. 1. Growth of food poisoning organisms on beef [illegible].

Keys to the Opposite Figure

Number	Line	Species of Organisms
1.		Staphylococcus 172
2.		Staphylococcus 178
3.		Staphylococcus 196
4.		Salmonella choleraesuis var. kunzendorf
5.		Salmonella paratyphi
6.		Salmonella enteritidis
7.		Salmonella gallinarum
8.		Salmonella schottmuelleri
9.		Salmonella typhimurium
10.		Salmonella pullorum
11.		Salmonella typhosa

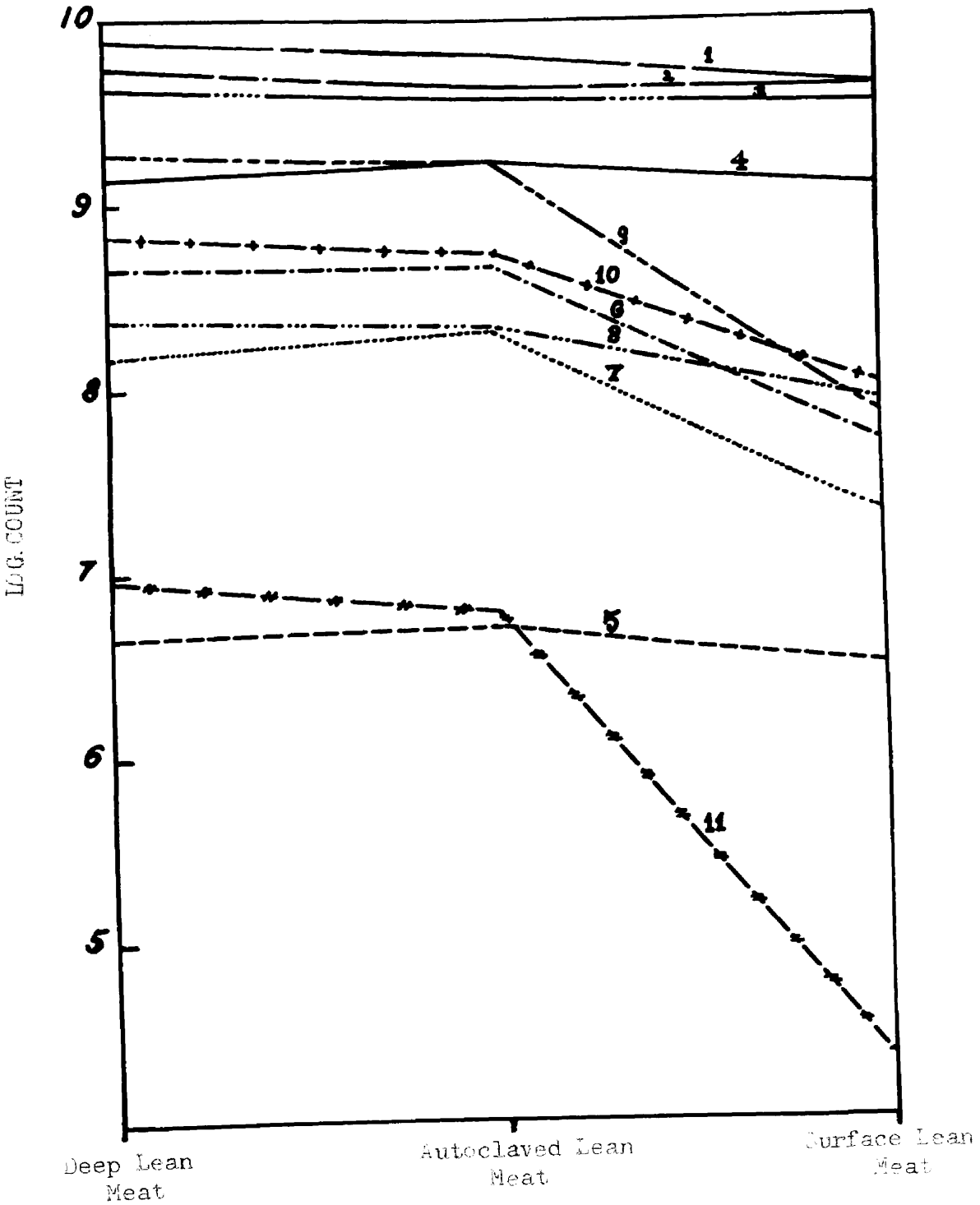

Fig. 2. Growth of food poisoning organisms on pork at 13.9 ± 2.8° .

Keys to the Opposite Figure

Number	Line	Species of Organisms
1.	———	Staphylococcus 172
2.	——·——	Staphylococcus 178
3.	——····——	Staphylococcus 196
4.	———	Salmonella choleraesuis var. kunzendorf
5.	— — — —	Salmonella paratyphi
6.	—·—·—·—	Salmonella enteritidis
7.	·····	Salmonella gallinarum
8.	—··—··—	Salmonella schottmuelleri
9.	— — —	Salmonella typhimurium
10.	—+—+—+—	Salmonella pullorum
11.	—*—*—*—	Salmonella typhosa

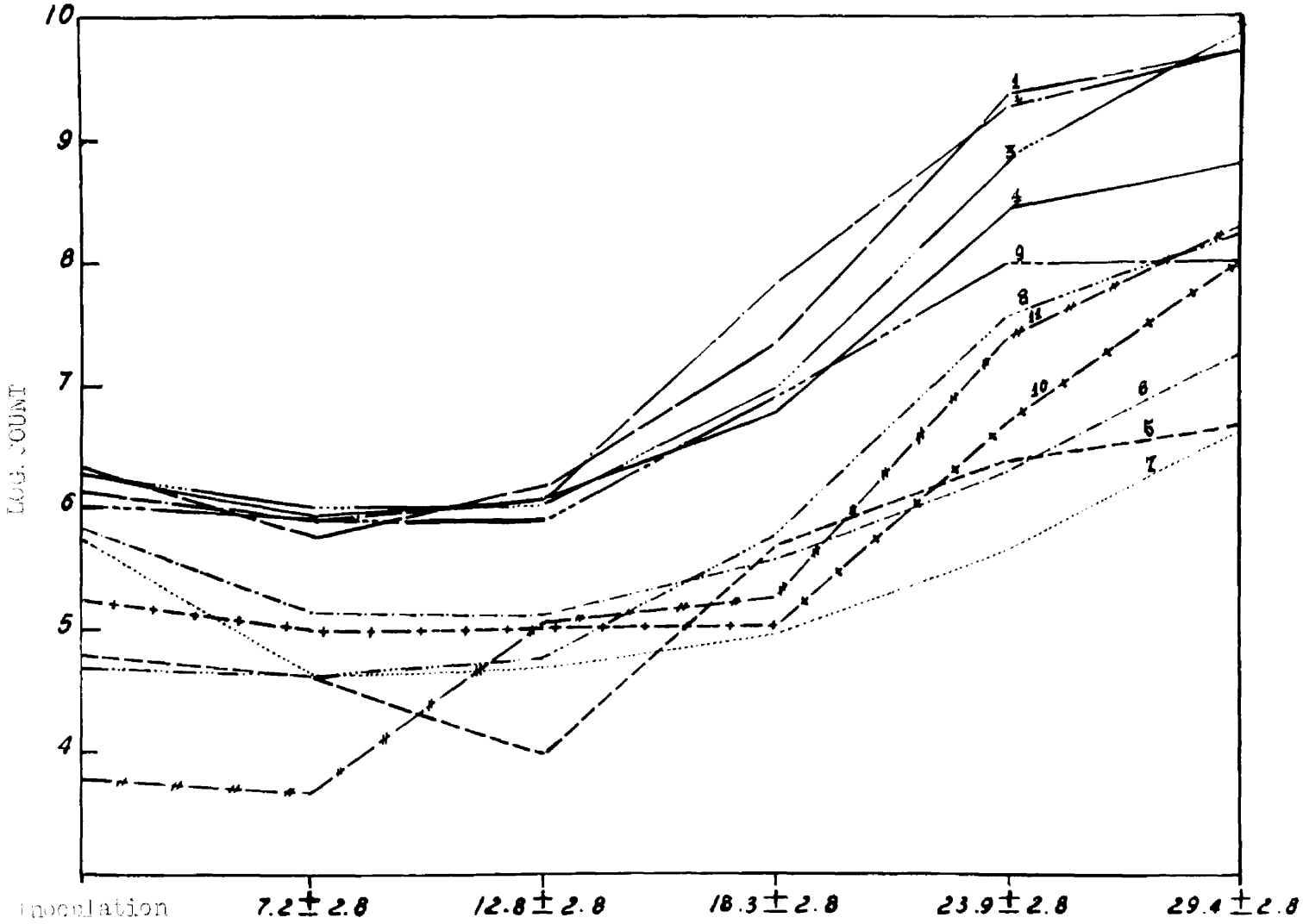

Fig. 5. Growth of food poisoning organisms on beef at various temperature ranges.

Keys to Opposite Figure

Number	Line	Species of Organisms
1.		*Staphylococcus* 172
2.		*Staphylococcus* 178
3.		*Staphylococcus* 196
4.		*Salmonella choleraesuis* var. *kunzendorf*
5.		*Salmonella paratyphi*
6.		*Salmonella enteritidis*
7.		*Salmonella gallinarum*
8.		*Salmonella schottmuelleri*
9.		*Salmonella typhimurium*
10.		*Salmonella pullorum*
11.		*Salmonella typhosa*

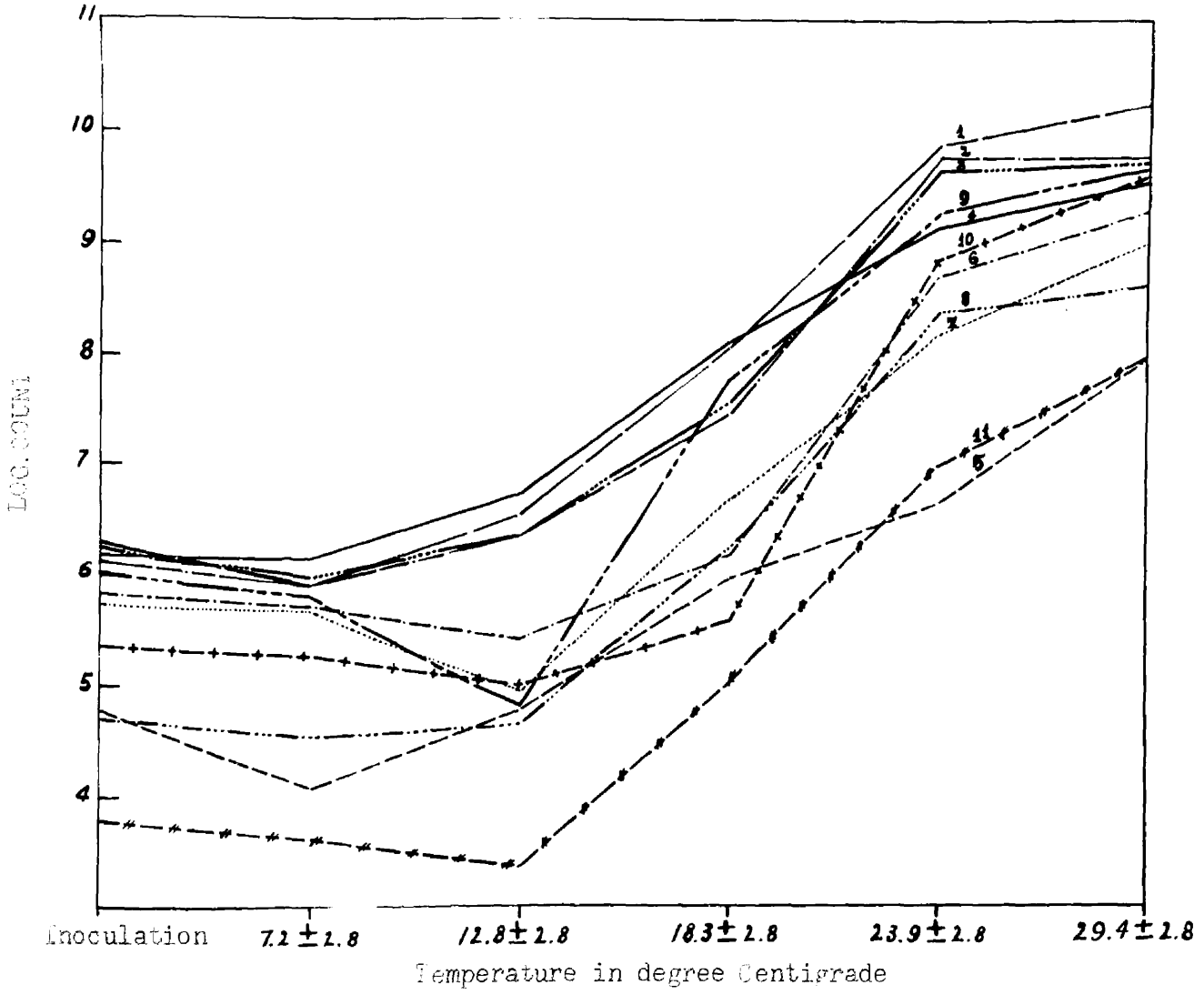

Fig. 4. Growth of food poisoning organisms on pork at various temperature ranges.

Keys to the Opposite Figure

Number	Line	Species of Organisms
1.		Staphylococcus 172
2.		Staphylococcus 178
3.		Staphylococcus 196
4.		Salmonella choleraesuis var. kunzendorf
5.		Salmonella paratyphi
6.		Salmonella enteritidis
7.		Salmonella gallinarum
8.		Salmonella schottmuelleri
9.		Salmonella typhimurium
10.		Salmonella pullorum
11.		Salmonella typhosa

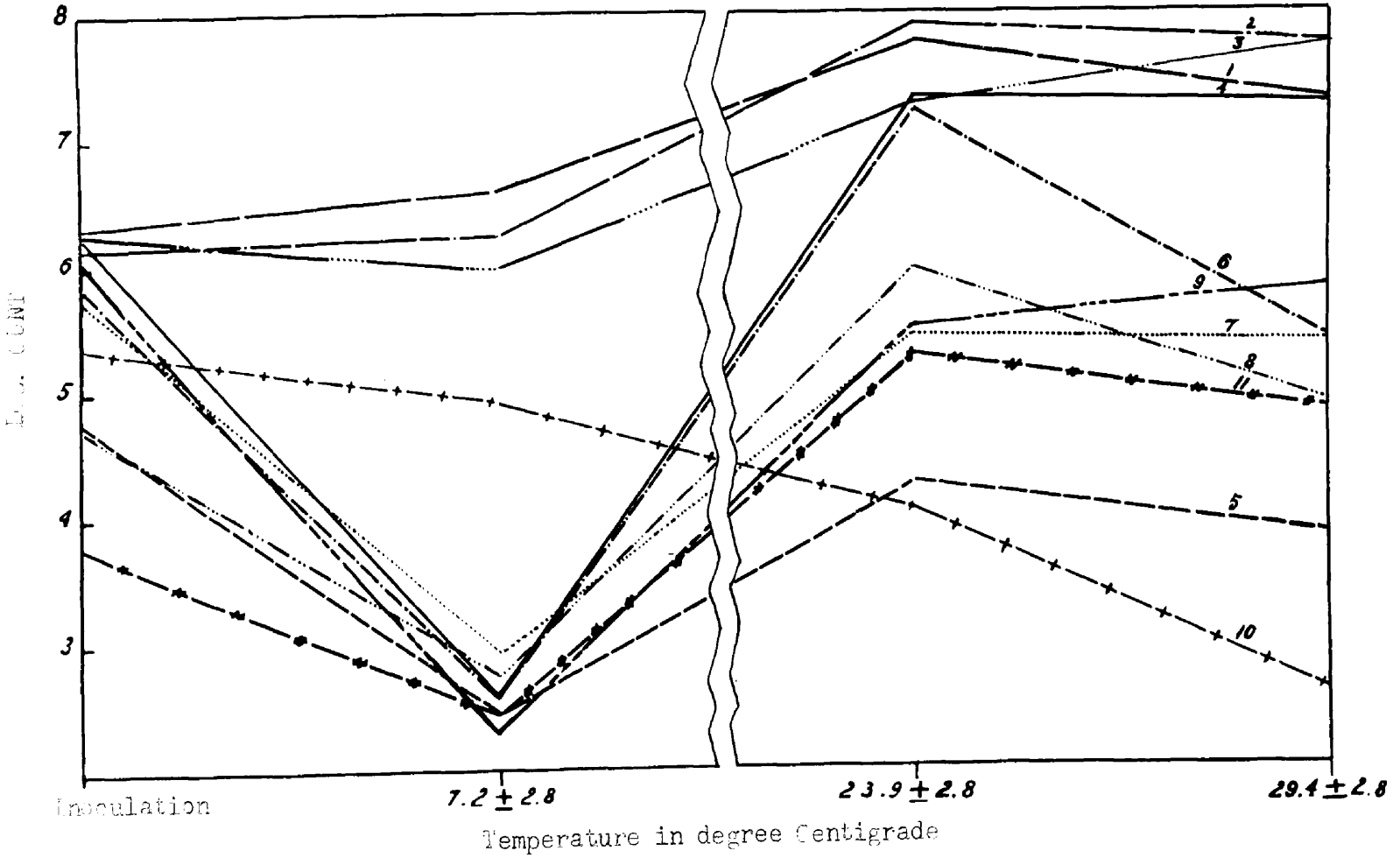

Fig. 5. Growth of food poisoning organisms on external fat of the beef carcass at different temperature ranges.

Keys to the Opposite Figure

Number	Line	Species of Organisms
1.		*Staphylococcus* 172
2.		*Staphylococcus* 178
3.		*Staphylococcus* 196
4.		*Salmonella choleraesuis* var. *kunzendorf*
5.		*Salmonella paratyphi*
6.		*Salmonella enteritidis*
7.		*Salmonella gallinarum*
8.		*Salmonella schottmuelleri*
9.		*Salmonella typhimurium*
10.		*Salmonella pullorum*
11.		*Salmonella typhosa*

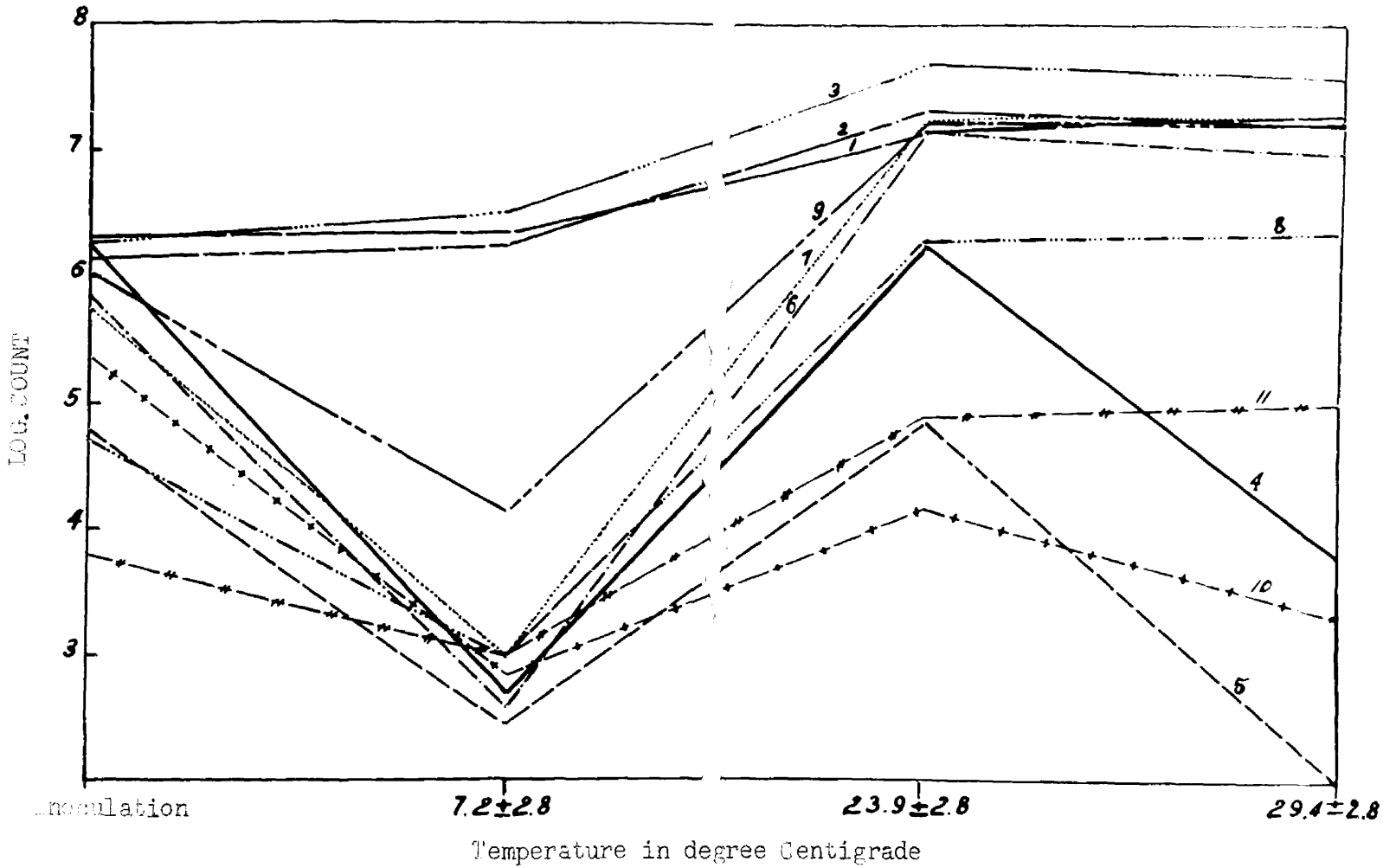

Fig. 6. Growth of food poisoning organisms on pork rind at different temperature ranges.

PART II. EFFECT OF ORGANIC ACIDS AND CHLORAMPHENICOL IN SANITIZING THE SURFACE OF BEEF AND PORK INOCULATED WITH FOOD POISONING ORGANISMS

Introduction

Experimental results obtained in Part I showed that the atmospheric temperature range, exclusive of a very high range such as 32.2-37.8 C (90-100 F) or a low temperature range such as freezing, optimal for growth of food poisoning staphylococci and salmonellae on lean pork and beef was 26.7-32.2 C (80-90 F). An experiment was, therefore, designed to find a chemical agent which would sanitize the surface of pork and beef inoculated with the food poisoning organisms and incubated at this optimum temperature range. Such a chemical agent in the concentration used should not be harmful to human beings, should not markedly alter the physical and chemical properties of the surface on the treated meat, and above all should be universally available and practical to use.

Experimental

As indicated on page 37 acetic acid was lethal to Staphylococcus aureus at 0.04 per cent concentration and to Salmonella aertrycke (Salmonella typhimurium) at 0.09 per cent (57).

Dehydroacetic acid inhibited S. aureus at 0.3 per cent and Salmonella pullorum at 0.3 per cent (97).

Chloramphenicol (chloromycetin) inhibited most staphylococci at 5.0 ug/ml and resistant strains of Salmonella typhosa and S. typhimurium at 5.0 ug/ml (60).

In this experiment chloramphenicol, sodium salt of dehydroacetic acid, acetic acid (in diluted 12 per cent or 120 grain vinegar), monochloracetic acid, dichloracetic acid, and trichloracetic acid were tested for their germicidal effects.

Repeated trials with various concentrations of the above organic acids showed that the sanitizing effect of the acids for the surface of pork and beef inoculated with the food poisoning organisms could be distinctly compared at 1 per cent concentration. A lower concentration than this allowed too much growth and above 1 per cent there was insufficient growth for comparison, especially with these acids which proved to be effective in reducing the counts of food poisoning organisms.

Chloramphenicol was used in 10 ug/ml concentration which was double that used to inhibit salmonellae and most staphylococci (60). This concentration also compared favorably in price with other chemical solutions used. Furthermore, a high concentration such as 25 ug/ml imparted some bitter taste to the meat which was treated with the solution.

Blocks of deep lean pork and beef (about 5 grams) were inoculated with 24 hour broth cultures of the food poisoning staphylococci and salmonellae as described in Part I. After inoculation the pork and beef were incubated at 30 C (86 F) for 90 minutes to allow the evaporation and absorption of the moisture in the inoculum on the surface of the meat.

This time interval also simulated the time elapsed between the killing and storing of pork and beef carcasses in the cooler.

After this short period of incubation of the inoculated pork and beef they were dipped in 1 per cent freshly prepared aqueous solutions of the acids used and 10 ug/ml chloramphenicol; each piece of meat was dipped in a separate sterile beaker containing the solution, removed immediately and placed in a separate sterile Petri dish which was slanted to provide drainage of the solution adhering to the meat.

The Petri dish containing the above inoculated and treated piece of meat was incubated again at 30 C (86 F) for 22 1/2 hours thus completing a 24 hour incubation period from the time of inoculation. At the end of the incubation period the piece of meat was dropped into a dilution bottle containing 10 to 100 ml of distilled water for the surface plating procedure as described in Part I.

The results of these experiments showed that 1 per cent acetic acid was the most effective of any of the solutions used in reducing the number of bacteria but still permitted a considerable number to grow. For this reason 2 and 4 per cent solutions of acetic acid were tried for sanitizing the meat. The 4 per cent solution proved effective as shown in table 9. The source of the acetic acid used in all experiments was distilled vinegar.

To determine any change in flavor due to 4 per cent vinegar, small and large retail cuts of pork and beef were dipped into it and allowed to drip at room temperature, 21.1-26.7 C (70-80 F), for from 1 to 4 hours before cooking for consumption in the regular manner.

Also to observe physical changes on larger pieces of pork and beef than those used for experimental work, chunks of beef and pork (about 30 to 100 grams) were dipped in 24 hour broth cultures of the food poisoning staphylococci and salmonellae. The inoculated pieces of meat were allowed to drip at room temperature for 90 minutes before dipping in the 4 per cent vinegar. These pieces of meat were next wrapped in pieces of moisture-vapor-proof meat wrapping paper or placed in closed Petri dishes for observation after standing 24 hours at room temperature. Control pieces were similarly inoculated and stored but not treated with the 4 per cent vinegar.

Results and Discussion

The strains of the food poisoning staphylococci used in these experiments were found to be much more resistant to the organic acids tested and to chloramphenicol than the food poisoning salmonellae studied, (tables and figures 7 and 8).

When used as dipping solutions for inoculated pork and beef 10 ug/ml chloramphenicol and 1 per cent trichloracetic acid were found to stimulate growth of the food poisoning staphylococci instead of suppressing them, (tables and figures 7 and 8). These solutions in these concentrations were obviously useless for the purpose of sanitizing meat.

The germicidal effect of the acids on the food poisoning staphylococci inoculated on the surface of pork and beef was in decreasing order: acetic, monochloracetic, dehydroacetic (sodium salt) and dichloracetic. The results are found in tables and figures 7 and 8.

Of the 8 species of salmonellae used S. choleraesuis var. kunzendorf and S. typhimurium inoculated on lean pork and beef were more resistant than the others to the germicidal action of the acids and chloramphenicol. The salmonellae, however, were not stimulated by 10 ug/ml chloramphenicol or by a 1 per cent concentration of the organic acids used. The germicidal effect of the various solutions on the food poisoning salmonellae on the pork and beef in decreasing order was: acetic, monochloracetic, dehydroacetic (sodium salt), dihydroacetic, trichloracetic and chloramphenicol. This germicidal effect on the salmonellae of 10 ug/ml chloramphenicol and of 1 per cent trichloracetic acid, however, did not differ very much, (tables and figures 7 and 8).

Acetic acid, therefore, was found to be the most effective of all solutions tested in reducing the numbers of food poisoning staphylococci and salmonellae on the surface of pork and beef. Monochloracetic acid, which also proved to be effective, can not be legally used in United States.

Four per cent vinegar reduced the numbers of the food poisoning staphylococci and salmonellae markedly. Seven of the 8 species of salmonellae inoculated on the surface of lean pork gave zero counts when 4 per cent vinegar had been used as a dipping solution. The remarkable germicidal power of acetic acid in this respect can be further appreciated if we consider that the pork had been inoculated with a very heavy inoculum which would not usually result from natural contamination; and that the inoculated meat had also been incubated at a very favorable temperature. In spite of the fact that inoculated lean pork which had

not been treated with a germicidal solution showed greater amount of growth of the food poisoning organisms than similarly inoculated and untreated lean beef, the numbers of bacteria killed on the pork appeared greater than those killed on the beef due apparently to the greater retention of the 4 per cent vinegar on lean pork than on lean beef, (table 9).

When used as a dipping solution 4 per cent vinegar did not impart any appreciable flavor to the pork or beef. Although a dilute solution of vinegar has been used as a tenderizing agent for some meats (100) no tenderizing effect was noted when pork and beef which had been treated with 4 per cent vinegar were consumed.

Larger pieces of lean pork (about 30 to 100 grams) which had been dipped in 4 per cent vinegar had a slightly whiter surface than normal after few hours. The larger pieces of lean beef dipped in the same solution also appeared whiter than normal unless they were wrapped in airtight material or where the treated surface lay flat against the bottom of a Petri dish in which case this portion retained its natural color; the surface exposed to the air in the Petri dish became whiter than normal.

This change in color also occurred in the larger pieces of pork and beef inoculated with the food poisoning staphylococci and salmonellae. Larger pieces of beef which had been inoculated with the food poisoning organisms but not treated with 4 per cent vinegar gave off offensive odors and darkened on the surface after 24 hours standing at room temperature. Those pieces similarly inoculated but treated with the vinegar

remained almost normal in odor and color; the color as just mentioned appeared normal on the surface coming in contact with an airtight wrapping material or the bottom of a Petri dish, (fig. C-3).

Both the inoculated and uninoculated lean pork whether treated or not with 4 per cent vinegar, emitted a foul odor and presented an unwholesome appearance after 24 hours standing at room temperature.

Summary

1. The food poisoning staphylococci used in these experiments were more resistant than the salmonellae to the germicidal action of dichloracetic acid, dehydroacetic acid (sodium salt), monochloracetic acid, and acetic acid.
2. A chloramphenicol solution of 10 ug/ml and a 1 per cent solution of trichloracetic acid were found to stimulate growth of the food poisoning staphylococci when used as dipping solutions in an attempt to sanitize the inoculated surface of pork and beef.
3. When used for sanitizing the surface of the inoculated pork and beef, the germicidal effect on food poisoning salmonellae of the 1 per cent organic acid solutions and of 10 ug/ml chloramphenicol was in decreasing order: acetic, monochloracetic, dehydroacetic (sodium salt), dihydroacetic, trichloracetic and chloramphenicol. The germicidal effect on salmonellae of 1 per cent trichloracetic acid and of 10 ug/ml chloramphenicol, however, was about the same.
4. The above decreasing order of germicidal effect also applied to the food poisoning staphylococci which had been inoculated on pork and

beef except that 1 per cent trichloracetic acid and 10 ug/ml chloramphenicol stimulated their growth.

5. Acetic acid was found to be the most effective of all the agents used to sanitize the inoculated lean pork and beef. Four per cent vinegar was found to be an effective and practical dipping solution to destroy the food poisoning staphylococci and salmonellae on the surface of pork and beef.
6. If the beef was wrapped in airtight material after dipping in 4 per cent vinegar there was no change of color due to this treatment. Furthermore, the treated beef kept longer due to destruction of microorganisms found on the surface.
7. The destruction of the food poisoning staphylococci and salmonellae on the surface of pork by 4 per cent vinegar appeared greater than that on beef due apparently to greater retention of the vinegar on pork. Pork treated with 4 per cent vinegar became whiter on the surface than normal.
8. Four per cent vinegar did not impart any appreciable flavor to the pork and beef which had been dipped in it.

Fig. C-3. Two pieces of beef removed from the same cut and inoculated with Salmonella enteritidis. The photographed surfaces had been kept in contact with the bottoms of Petri dishes.

Left - control
Right - treated with 4 per cent vinegar

Table 7

Growth of food poisoning organisms on deep lean beef treated with 1 per cent acid solution or 10 ug/ml chloramphenicol (chloromycetin) after 24 hour incubation at 30 C. Counts in millions.

Organism	Control	Chloramphenicol	*Acid 1	Acid 2	Acid 3	Acid 4	Acid 5
Staphylococcus 172	5,300.000	11,000.000	8,400.000	910.000	7.400	2.800	.620
Staphylococcus 178	5,400.000	5,600.000	14,000.000	340.000	37.000	4.100	.480
Staphylococcus 196	9,600.000	34,000.000	36,000.000	10.000	21.000	14.000	.060
Salmonella choleraesuis var. kunzendorf	630.000	660.000	310.000	35.000	11.000	.820	.004
Salmonella paratyphi	4.400	4.200	.012	0	.040	0	.0001
Salmonella enteritidis	16.000	1.600	.340	.009	.150	.009	.0002
Salmonella gallinarum	4.000	.500	.620	.400	.220	.011	.010
Salmonella schottmuelleri	170.000	48.000	.100	1.000	.810	0	0
Salmonella typhimurium	100.000	37.000	26.000	100.000	.780	.010	0
Salmonella pullorum	100.000	1.100	0	.002	.011	0	.009
Salmonella typhosa	140.000	1.000	.080	.0003	.0005	.001	0

* Acid 1 - Trichloracetic acid, Acid 2 - Dichloracetic acid, Acid 3 - Dehydroacetic acid (sodium salt), Acid 4 - Monochloracetic acid, Acid 5 - Acetic acid.

Table 8

Growth of food poisoning organisms on deep lean pork treated with 1 per cent acid solutions or 10 ug/ml chloramphenicol (chloromycetin) after 24 hour incubation at 30 C. Counts in millions.

Organism	Control	Chloramphenicol	*Acid 1	Acid 2	Acid 3	Acid 4	Acid 5
Staphylococcus 172	18,000.000	15,000.000	11,000.000	9,400.000	2,300.000	2,500.000	48.000
Staphylococcus 178	6,200.000	20,000.000	15,000.000	2,300.000	300.000	48.000	27.000
Staphylococcus 196	5,400.000	19,000.000	14,000.000	3,400.000	3,800.000	5.000	7.900
Salmonella choleraesuis var. kunzendorf	3,300.000	110.000	87.000	5.600	.860	.680	1,100.000
Salmonella paratyphi	82,000.000	.110	.420	.300	.045	0	0
Salmonella enteritidis	1,900.000	14.000	.019	.530	.002	.230	.023
Salmonella gallinarum	940.000	7.600	.260	.019	.500	.001	.210
Salmonella schottmuelleri	430.000	100.000	.004	.034	.070	.001	.007
Salmonella typhimuerium	4,900.000	2,400.000	2,600.000	2,300.000	230.000	150.000	4.300
Salmonella pullorum	3,600.000	13,000	22.000	7.000	.001	.005	1.000
Salmonella typhosa	80.000	.870	.017	0	0	0	.001

* Acid 1 - Trichloracetic acid, Acid 2 - Dichloracetic acid, Acid 3 - Dehydroacetic acid (sodium salt), Acid 4 - Monochloracetic acid, Acid 5 - Acetic acid.

Table 9

Growth of food poisoning organisms on deep lean beef and pork treated with 4 per cent acetic acid and incubated 24 hours at 30 C. Total counts.

Organism	Beef		Pork	
	Untreated	Treated	Untreated	Treated
Staphylococcus 172	5,300,000,000	2,000	18,000,000,000	1,000
Staphylococcus 178	5,400,000,000	43,000	6,200,000,000	12,000
Staphylococcus 196	9,600,000,000	8,300,000	5,400,000,000	13,000
Salmonella choleraesuis var. kunzendorf	630,000,000	310,000	3,300,000,000	0
Salmonella paratyphi	4,400,000	0	82,000,000,000	0
Salmonella enteritidis	16,000,000	1,800	1,900,000,000	0
Salmonella gallinarum	4,000,000	43,000	940,000,000	0
Salmonella schottmuelleri	170,000,000	100	430,000,000	0
Salmonella typhimuerium	100,000,000	7,500	4,900,000,000	3,000
Salmonella pullorum	100,000,000	0	3,600,000,000	0
Salmonella typhosa	190,000,000	100	80,000,000	0

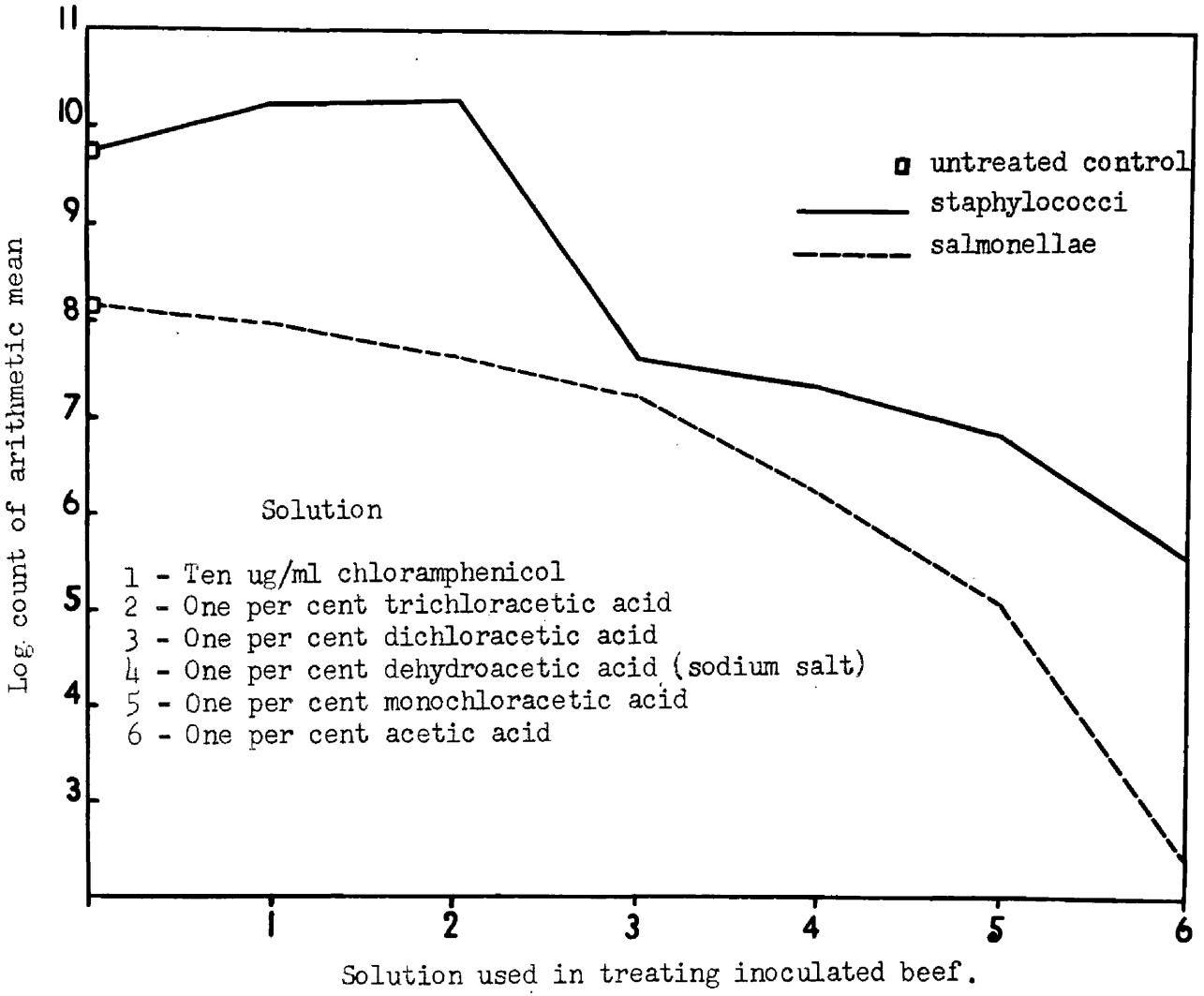

Fig. 7. Growth of food poisoning organisms on deep lean beef treated with one per cent acid solutions or 10 ug/ml chloramphenicol (chloromycetin) after 24 hour incubation at 30 C.

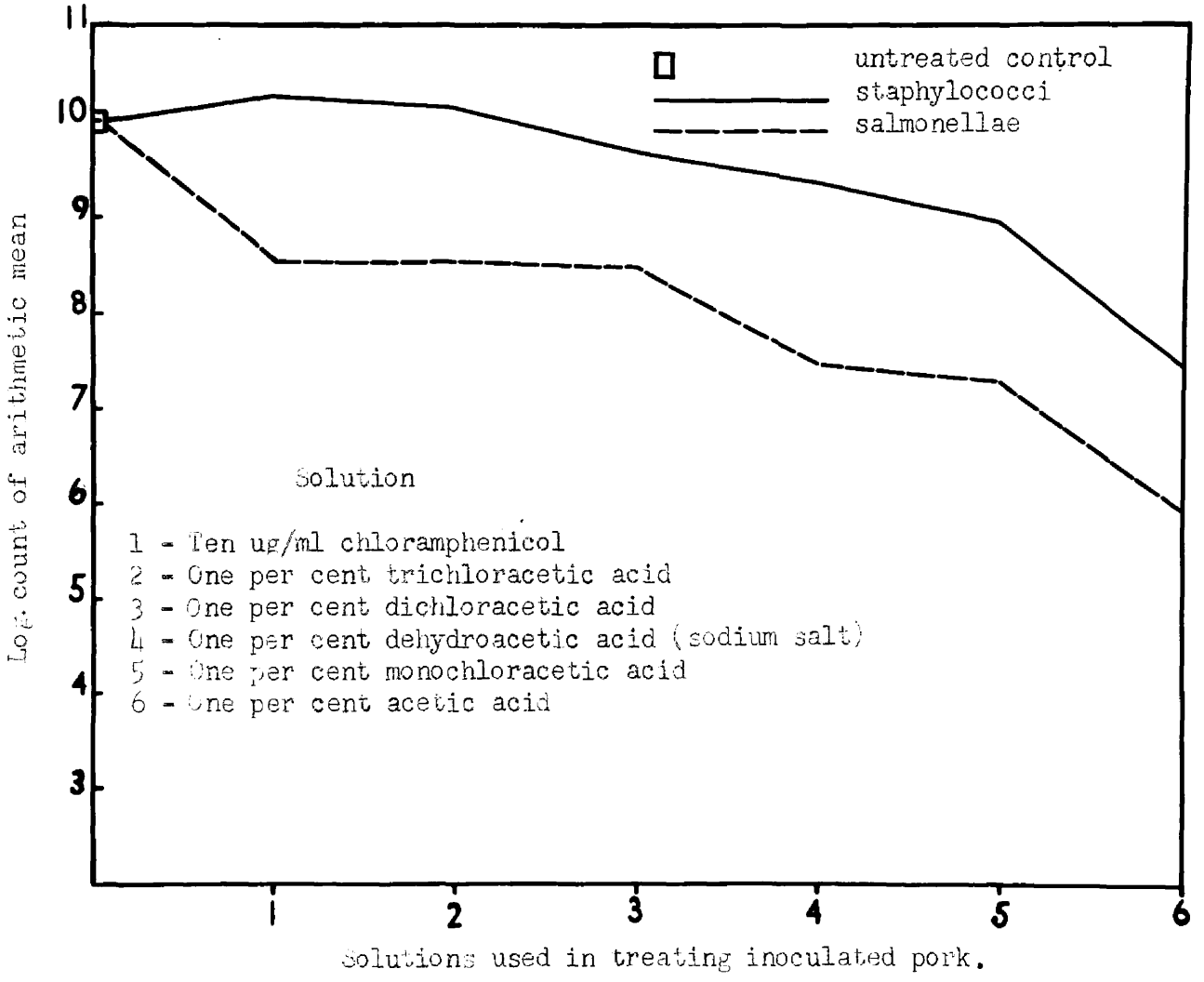

Fig. 8. Growth of food poisoning organisms on deep lean pork treated with one per cent acid solution or 10 ug/ml chloramphenicol (chloromycetin) after 24 hour incubation at 37° C.

GENERAL DISCUSSION

The results of the study on the growth of food poisoning staphylococci and salmonellae on the surface of raw lean pork and beef showed that these meats were favorable media for the growth of the organisms. At a room temperature of 21.1-26.7 C (70-80 F) and at 26.7-32.2 C (80-90 F) these organisms increased to large numbers on the surface of lean pork and beef during 24 hours, (tables 3 and 4). The surface moisture of the meats and the humidity of the air also determined the bacterial growth to a great extent; if the surface of lean pork and beef was drier than normal due to a high temperature of 26.7-32.2 C (80-90 F) together with a low humidity the growth would be less than at an even lower temperature of 21.1-26.7 C (70-80 F) together with a high humidity. A refrigerating temperature of 4.4-10.0 C (40-50 F) was effective in preventing the growth of food poisoning staphylococci and salmonellae on the surface of lean pork and beef for at least 24 hours. Also, enterotoxin was not produced by food poisoning staphylococci at this temperature.

To determine the numbers of bacteria on the surface of raw pork and beef was a difficult problem. A swabbing method, used by Mallmann and Churchill (61) to remove bacteria from a four-inch square area on the surface of the beef quarter with a sterile moist cotton swab, showed very marked variations in bacterial counts. The authors stated that this variation was due to the irregularity in the contamination of the carcass and variability in contamination of the various carcasses. They believed

that the method undoubtedly failed to remove all the organisms but it gave comparable results, was simple and involved less labor and equipment.

While the above swabbing method may be well applied for an investigation which requires only approximate numbers of bacteria it was found unsatisfactory for a comparison of the growth of food poisoning staphylococci and salmonellae on the surface of lean pork and beef at various temperature ranges and for comparison of the germicidal effect of some organic acids and chloramphenicol on the growth of these organisms on lean pork and beef. Variability in the surface moisture and adhesiveness on different parts of the pork and beef carcasses and even on the same cuts contributed to the variation in numbers of bacteria which were obtained by the swabbing method. Furthermore, the surfaces of pork and beef were irregular.

To recover the organisms which had been inoculated on the surface of raw lean pork and beef and their coverings for plating after 24-hour incubation, it was found that there was less variation in numbers of bacteria when the inoculated pieces of pork and beef were shaken in the desired dilution of distilled water in dilution bottles to remove the bacteria.

Pork rind and external fat of the beef carcass were poor media for the growth of food poisoning staphylococci and salmonellae in comparison to the lean pork and beef, (tables 5 and 6).

To determine the number of salmonellae which had been inoculated on raw pork and beef after 24 hour incubation was another difficult problem due to the presence of other organisms on the meats. Segalove

and Dack (80), when they studied the growth of bacteria associated with food poisoning, experimentally inoculated into dehydrated meats, autoclaved all their dehydrated pork and beef samples in airtight containers before inoculating with salmonellae. This heat treatment doubtless altered the chemical composition of the original materials as well as destroyed all microorganisms present on them; both of these factors might influence the growth of the inoculated bacteria. However, they worked with pork and beef which had been dehydrated and also salted, therefore, the composition of such meats approached that of cooked meats in comparison with the unsalted fresh market meats. The original bacterial load of such dehydrated and salted meats was also lower than that of fresh market meats.

The results of the experiments in the present study showed that there was no significant difference between the growth of food poisoning staphylococci and salmonellae on raw lean pork and beef taken 1 cm beneath the surface and on cooked lean pork and beef when incubated at 21.1-26.7 C (70-80 F). However, there was less growth of salmonellae inoculated on the surface lean pork and beef taken from the regular retail cuts due to the antagonism offered by other organisms present, especially E. coli, and to the evaporation of surface moisture.

Since the study was to determine the growth of food poisoning staphylococci and salmonellae on raw pork and beef at various temperature ranges and also to control the organisms on raw pork and beef by the use of chemical agents all meat samples used in the experiments were not autoclaved.

S. S. agar which proved to be excellent in differentiating colonies of salmonellae from other organisms by the streaked plate was found to be unsatisfactory for making the regular poured plate for bacterial count as far as the differentiation was concerned. Since the usual streaked plate method could not be used for counting bacteria a surface plating method was developed to assure differentiation, separation, and uniformity in size of colonies of salmonellae on S. S. agar. Chapman-Stone medium was similarly used as a selective medium which permitted counting of staphylococcus colonies with gratifying results.

Pork was found to be a better medium for the food poisoning organisms than beef. Food poisoning staphylococci exceeded salmonellae in growth on lean pork and beef and their coverings and also exceed them in their resistance to the organic acids tested and to chloramphenicol.

At 1 per cent concentration acetic acid was found more effective than monochloracetic acid, dehydroacetic acid (sodium salt), dichloracetic acid, and trichloracetic acid in destroying the food poisoning organisms on pork and beef. The 1 per cent solution of acetic acid was also more effective than 10 ug/ml chloramphenicol in this respect. Considerable numbers of organisms, however, remained viable after treatment with 1 per cent acetic acid.

Four per cent vinegar was much more effective than 1 per cent acetic acid in destroying the food poisoning organisms inoculated on pork and beef. The solution did not impart any appreciable flavor to the pork and beef.

Beef treated with 4 per cent vinegar retained the natural color longer and kept longer if wrapped in airtight material due to destruction of many microorganisms found on the surface.

GENERAL CONCLUSION

Pork and beef are good media for food poisoning staphylococci and salmonellae which increased to great numbers at 15.6-32.2 C (60-90 F) in 24 hours.

A refrigerating temperature of 4.4-10.0 C (40-50 F) in the household refrigerator effectively suppresses the growth of the food poisoning organisms for at least 24 hours.

Whenever possible pork should be kept and handled with the rind and beef with the external fat of the beef carcass intact because these natural coverings are poor media for the growth of the food poisoning staphylococci and salmonellae.

Fresh pork and beef should be dipped in four per cent vinegar before keeping in a cold storage or displaying for sale. This treatment is practical and effective in destroying the food poisoning staphylococci and salmonellae that may contaminate the surface of the meats. The vinegar does not impart any appreciable flavor to the meats.

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