MICROBIOLOGICAL PROBLEMS IN THE PRESERVATION OF MEATS

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CONTENTS

Until some sixty years ago, man depended largely upon the perishable food supplies produced within a few hundred miles of his home. In general, the methods of preparation and the kinds of foodstuffs of animal origin had changed little since ancient times. With the rise of bacteriology, coupled with developments in the physical and chemical sciences, procedures have been elaborated for the control and study of meat foods. However, the pace of all historical development teaches us that the work in meat technology, as in other fields of food technology, has just begun.

For the preservation of meats, the following practical methods have been developed, some of them with an empirical background of great antiquity: partial drying and dehydration; curing with salt, nitrates, nitrites, and sugars; smoking, icing and refrigeration; culinary heating; packing in metal and glass containers. The maintenance of good standards of quality as well as of sanitation in the products often depends on adequate microbiological control; and, on occasion, specific microbiological problems are presented which depend, for an ultimate solution, upon fundamental research.

Practically every process employed in the meat industry hinges upon the reduction and control of microbiological activities. The proteins, carbohydrates and fats of meat foods are all vulnerable to the action of many kinds of microorganisms. There are numerous species of nonpathogenic bacteria, yeasts, and

molds which can discolor fresh and cured meats by oxidative processes; induce oxidative rancidity in fats, and discolor fats and oils with their pigments; ferment cured meats and canned meats with carbon dioxide production; and cause spoilage of unprotected proteins in a number of ways. For discussion of the many activities of microörganisms in meat foods and practical measures for control of microörganisms in meat plants, the reader is referred elsewhere (36).

The following sections illustrate a few of the problems arising from large-scale processing of meat foods which may be of general bacteriological interest.

1. ACTION OF CERTAIN BACTERIA ON MYOHEMOGLOBIN AND NITRIC-OXIDE DERIVATIVES FORMED IN CURED MEATS

The color of muscle tissue is red in the fresh, unheated state, but this color is not due to residual blood or hemoglobin. The pigment is myohemoglobin (or myoglobin), an integral part of the muscle tissue, which does not circulate in the blood stream. Hektoen, Robscheit-Robbins, and Whipple (27), by means of the precipitin reaction, have demonstrated the non-identity of myoglobin and hemoglobin. However, the general properties of these two pigments are sufficiently alike so that one may use the blood pigment for certain experiments in place of the muscle pigment without serious error in the conclusions drawn therefrom, according to Urbain and Jensen (103).

Unlike fresh meats, the cured meats retain their redness on cooking. Chiefly through the work of Haldane (25) and Hoagland (29), the stability of the cured meat pigment towards heat has been shown to be a property of nitric-oxide derivatives of the pigment resulting from the chemical interaction with nitrite either added directly in the curing salts or produced through bacterial reduction of nitrate. (These investigators assumed the pigment of meat to be identical with the blood pigment.) Successful curing of the meat eventually leads to the formation of nitric-oxide myohemoglobin, according to the reaction: NO + myohemoglobin = nitric-oxide myohemoglobin. The source of the nitric oxide is nitrite or more properly nitrous acid. Upon heating, the nitric-oxide myohemoglobin is converted to nitric-oxide hemochromogen, a denatured protein. The fact that this hemochromogen is red is one of the objects gained by the employment of nitrite and nitrate in the curing of meats. Any reaction which yields unusual colors in cured meats is undesirable as a matter of custom rather than of sanitary deficiency.

In the past, one of the most troublesome phenomena in the meat plant was the discoloration of cured meats, such as green rings in frankfurters, green boiled hams, and dried beef hams. The pink nitric-oxide pigments are sensitive to oxidations of microbial origin and become emerald green, gray, and brown in color when "oxidase"-forming bacteria act upon them. Reducing agents can often restore the pink color but the use of compounds, such as sulfites, is prohibited by law. The greening action of a few species of bacteria on blood agar media is so characteristic that considerable diagnostic importance has been attached to the phenomenon. The green pigment formed has been ascribed to a variety of

causes, among which are the following: formation of methemoglobin, Zinsser and Bayne-Jones (115), and Valentine (104); action of lactic acid, Ruediger (87) and Hagan (24); oxidation, McLeod and Gordon (71); reduction, Holman (31); xanthroproteic reaction, Barnard and Gowen (4); and an optical illusion, Boxer (7).

Jensen and Urbain (51) have shown that many species of bacteria possess mechanisms for discoloring blood pigments and their nitric-oxide derivatives. They found that the kinds of microörganisms responsible for the formation of green pigments in meats and blood-agar are those which oxidize hemoglobin, nitric-oxide hemoglobin, nitric-oxide hemochromogen, and hematin. In addition, bacteria producing hydrogen sulfide cause green discolorations of these pigments. The role of hydrogen sulfide in the production of these discolorations is one that is somewhat involved. Hydrogen sulfide reacts with reduced hemoglobin to form a purple compound. On exposure to oxygen, this purple compound oxidizes rapidly to form a green compound. Spectrophotometric measurements have been made on the green pigments obtained by the action of certain oxidizing bacterial enzymes on nitric-oxide hemoglobin and by the action of hydrogen sulfide on hemoglobin. These compounds were found to be spectroscopically different from methemoglobin. It will be remembered that methemoglobin is brown in color, not green. Hence, in speaking of the greening action of some bacteria on blood-agar plates, one should not designate the greenish halos as due to methemoglobin. Recent work indicates that these green pigments or "verdohemes" may be related to, or identical with, some of the bile pigments like biliverdin. The reader is referred to the publications of Lemberg and collaborators (61 to 67) for information on the chemistry and occurrence of some of these green pigments in various biological materials.

Green pigments, prepared by treating fresh ground beef myoglobin and fresh defibrinated hog's blood with H_2O_2 and $NaNO_2$ and also with H_2S, were fed to 25 white rats and 25 white mice, supplementing 20% of the green pigments in the standard basal diet. Each lot of test animals was fed these pigments for one month with no ill effects. These test animals were irradiated each week for 30 minutes under a General Electric S2 ultraviolet lamp; and they exhibited no untoward reactions to this treatment, i.e., there was no photo-sensitization induced by porphyrins as described by Blum (6).

What are commonly spoken of as "green rings" in sausage occur near the casing, varying in frankfurters, for example, in distance from the casing from one-sixteenth to one-eighth of an inch (1.5 to 3 mm), and in width from a faint—at times, discontinuous—circle to a band one-eighth of an inch (3 mm) across. The green discoloration may appear also as a core in the center of the sausage. The circular form of green ring has often been noted in square-pressed bologna. The various bacteria which can bring about these oxidizing reactions are: (a) those which elaborate oxidizing enzymes, some insensitive to catalase and thermostable, and some sensitive to catalase and thermolabile (effects destroyed by heating to 155–160F (68.4–71.1C) for 15 minutes); (b) H_2S-forming bacteria, both aerobes

and facultative anaerobes growing at an optimum zone in the sausage; (c) "oxidizing" bacteria growing at an optimum zone only. The ring formation is probably due to a phenomenon similar to that observed by Neill and Hastings (76) in which the oxygen tension governs the amount of oxidized hemoglobin in the presence of a constant amount of pneumococcus extract. Their data show that when the oxygen tension approaches zero, only traces of the oxidized pigment are formed by the extract; and when the tension of molecular oxygen is great, again only traces of the oxidized pigment are formed. However, at intermediate, optimum oxygen tensions much oxidized pigment is formed. In many instances, the green-ring formation in casing sausage is formed according to the quantity of oxygen dissolved, provided that the bacteria have grown sufficiently in the trimmings from which the sausage was made. The addition of sterile bacterial filtrates to fresh sausage materials has resulted in the production of green rings in the finished product. For a detailed discussion of these green rings, the reader is referred elsewhere (38).

The iridescence of sliced cured meats, such as ham, bacon, and dried beef, is due to the peculiar surfaces of cured meat fibers. The color is a structural color as opposed to a pigment color. It is due to the breaking up of white light by the highly fibrous character of the surface and to the film of fat on these fibers. If the fat is removed, the iridescence disappears. Any oil applied to the defatted surface will restore iridescence. The phenomenon has no sanitary significance, and is related to that of the diffraction grating or "clam-shell" play of colors.

2. Ante-mortem and post-mortem changes of tissues of food animals

(*a*) *Bacteriology of Tissues of Living and Slaughtered Hogs.* There is very little information available as to the occurrence of bacteria in tissues and blood in healthy persons, according to Topley and Wilson (100). However, some writers (Norris and Pappenheimer (77), Arnold (3), and Burn (11)) state that there is definite evidence that tissues may not always be sterile in normal human beings. Most of the early work on the bacteriology of tissues of normal animals indicates that tissues, especially muscle, are sterile. Other workers have reported the presence of a variety of microörganisms in the blood, viscera, and muscular tissues of healthy animals (48). In 1926 Reith (82), after a careful study of various tissues of hogs and laboratory animals, came to the conclusion that aerobes and anaerobes are present in the musculature and blood of apparently normal animals. If one upholds the ante-mortem infection theory, the problem of meat spoilage becomes a refrigeration-engineering problem to a large extent, and whatever spoilage occurs must be considered unavoidable even though applied bacteriology functions ideally. However, the work of Burn (11) on post-mortem bacteriology points to the phenomenon of agonal invasion; and the work of Jensen and Hess (48) supports the view that the invasion of bacteria is agonal and post-mortem rather than ante-mortem. A series of biopsy studies was conducted on prime normal hogs whose blood, bones, bone marrows, and muscle tissues were examined bacteriologically. After the surgical fields were closed, sutured, and heavily covered with celloidin, the animals were immediately taken to the killing floor,

hoisted, stuck and bled, washed, dehaired, butchered, and dressed. Before they were chilled and tanked, the control tissues and expressed blood were examined (40 minutes later). Except for one animal (which appeared normal, but harbored *Hemophilus sp.* in all tissues examined while alive and after dressing as well), none of the hogs, while alive, showed the presence of microörganisms in the tissues or the blood. The post-mortem findings indicated, however, that bacteria may occasionally be found in the tissues and blood. The tissues post-mortem may show the presence of species of *Achromobacter, Pseudomonas, Serratia, Bacillus, Proteus, Micrococcus, Clostridium,* "diphtheroids," and *Torula* (48). These findings suggest that bacteria may on occasion be found in tissues immediately after death from slaughter.

Many investigators have determined a few of the mechanisms which remove bacteria from the blood stream, a subject which has been reviewed elsewhere (48). Norris and Pappenheimer (77), who introduced *Serratia marcescens* into the oral cavity of human beings immediately after death, recovered these bacteria from the lungs several hours later. Some pathologists say that contamination of the heart's blood occurs through the large veins from the lungs, and that heart's blood at autopsy practically always shows contamination with many species of microörganisms. It has long been known by pathologists that, at autopsy, bacteria and remnants of food may be found in the lungs, i.e., "food down the wrong way." It is not uncommon to find corn and other food in the lungs of slaughtered hogs. Hülphers (33) describes microörganisms found in lungs of slaughtered hogs, and most of these microörganisms appear to be soil flora. There is a unique mechanism of contamination of some living animals shown first by Tarozzi (96) and later by Canfora (12). Each of them injected spores of *Clostridium tetani* into animals. There were no ill effects from these inoculations. After a considerable length of time (up to 55 days), a bone of one of the animals was broken or traumatized, following which tetanus usually occurred, showing the longevity of spores in the normal animal body. Koser and McClelland (56) found the spores of clostridia recoverable from the body long after the spores of aerobes had disappeared. Contamination of the bone marrows, especially the red marrows, may perhaps occur in the normal animal through permeability of the intestinal mucosa, via wounds, the lungs, and the upper respiratory tract. In this connection, the occurrence of latent infections should also be considered, Meyer (73).

Many theories, current in the meat-processing industry, on the source of contamination of tissues have been investigated (48), and brief mention is made of some experiments which may be deserving of further study. The skin of a hog obviously is heavily contaminated with microörganisms. When the stick knife passes through the skin, severing the jugular vein and sometimes the carotid artery, the blade is washed with venous and often with arterial blood. The heart may beat from two to nine minutes after the stick wound is made. Some of the shackled, hoisted hogs contract their heads in the direction of their forelegs and thus withhold some of the blood by constriction and hematoma, allowing some blood from this area to reach the heart and be circulated. A "negative" pressure

may be set up in the severed or pierced vessels, owing to the labored breathing accompanying exsanguination (oxygen starvation). The flow of the pooled blood and blood within the vein is towards the heart.

Tests were made by dipping the blades in cultures of various bacteria and then sticking the hogs in the usual manner. Some of these bacteria (possessing an identifying cultural characteristic to distinguish them from the usual flora) were recovered from the marrow of the tibia and other bones. A large number of hogs were then bled aseptically (a technic which obviously brings to mind the methods employed by the serum companies which produce hog-cholera serum and other immunizing serums. It has long been observed that such "serum" hogs when slaughtered produce a very low incidence of sour meats). However, the aseptic bleeding as effected in the above tests did not close all portals of contamination of the tissues, including bone marrow; but technical sanitation of the pork-block in conjunction with other methods of applied bacteriology, reviewed elsewhere (48), effected a marked reduction in the numbers of sour marrows and tissues.

Practically all investigators in this field have observed the absence of coliform bacteria in hams. Boyer (8) states: "The absence of the *Bacillus coli* group of organisms from the numerous cultures taken from these hams is of particular interest. The members of this group are abundant and ubiquitous on the killing floor, and are almost invariably found on the surfaces of the carcasses which are exposed during killing floor operations. Their absence is of special significance in that it goes far to eliminate the possibility that organisms present in the hams gain access during killing floor operations."

The stick-blade contamination and the train of conditions following the severing of the neck vessels should favor the entry of coliform and other bacteria into the blood and marrow. However, some factor prevents their reaching the marrow or surviving therein if they do reach it. It is well known that undiluted, fresh mammalian blood is bactericidal (1). Hog's blood was drawn aseptically from the tail of a live hog, as is done by the commercial producers of serum, and bled directly into sterile containers containing glass beads for defibrination. Various freshly isolated strains of bacteria (*Escherichia coli*, species of *Pseudomonas*, *Serratia*, *Achromobacter*, vegetative clostridia and *Bacillus*, and *Staphylococcus aureus*) were added in minimum amounts of menstruum so that the blood would contain about 50,000 viable cells per milliliter. The defibrinated blood containing these bacteria was cultured at short intervals up to 24 hours. It was found that many of the flasks were practically sterile after 2 to 5 hours and other strains survived with less complete reduction in numbers. The ham-souring types of bacteria, such as certain strains of *Serratia*, *Achromobacter*, *Clostridium putrefaciens* of McBryde, and *Pseudomonas*, were more or less resistant to the bactericidal effects of hog's blood. The suspensions of *Staphylococcus aureus* and *Escherichia coli* were often sterile after 2 to 5 hours. These studies should be repeated and extended.

(*b*) *Autolysis of Tissues.* Over 70 years ago Hoppe-Seyler (32) recognized liquefaction of dead tissues occurring without accompanying putrefaction, and he noted that the phenomenon resembled the effects of digestive ferments. Some

experiments performed by Ernst Salkowski (88) in 1890 led this investigator to the discovery of tissue autolysis, which he named "autodigestion." He used chloroform as a bacteriostat. Little notice was taken of this work for a decade until Martin Jacoby (35) took up the work again and introduced the term "autolysis."

The one great difficulty in evaluating true autolysis is the uncertainty of continuing and effective antisepsis. Von Fürth (107) states that it was assumed there would be no danger of bacterial growth in finely divided tissues saturated with chloroform or toluol. He states that it was usually supposed that, if a few drops of toluol or a few bits of thymol were added to a thick emulsion of some tissue, it would be quite safe to incubate the emulsion for months, and that the danger of bacterial action "would be surely and for all time eliminated by such a purely symbolic performance (for that is actually about all it represents).... It is quite obvious why 'discoveries' prospered and multiplied in our literature on the same scale as did the bacteria in the pots and jars of the experimenters."

The question of tissue sterility also enters into the picture. There are times when many tissues of slaughtered animals are not sterile, although biopsy materials are usually sterile.

A large number of experiments have been performed to demonstrate the native autolytic enzymes of tissues (109). Some microbiologists do not subscribe to the existence of autolytic enzymes because of the faulty bacteriological techniques employed in certain biochemical experimentation. Recent work by Reeves and Martin (81) demonstrates that bacteria do grow in many tissue preparations containing preservatives calculated to stop bacterial growth. In their experiments, the sterility of digests could be determined only by smears and cultures. They repeatedly found bacteria growing in digests which were free from putrid odors and showed no evidence of bacterial growth. However, there are methods (2) available for the isolation of autolytic enzymes. Further work is needed in this field with new preservatives and more adequate microbiological controls.

There are several opinions extant on the exact mechanisms responsible for the tendering of meat. Some observers believe that the connective tissues are responsible for tough meat and that the change from collagen (of connective tissues) to gelatin correlates closely with the degree of tenderization. Other workers have concluded that connective tissues are very little changed and that the tenderness of meat is due to the muscle fibers becoming inelastic and thus tender. Another group does not consider that autolysis by native ferments plays any appreciable role in meat tendering during the first month of storage and does not reckon that microörganisms aid in the process.

The question of autolysis has been discussed in detail by Hoagland, McBryde, and Powick (30) who believe that meat tendering during storage may be regarded as largely due to enzyme action. There is no question that some denaturation of soluble protein occurs during rigor mortis. Denaturation, as well as autolysis of muscle, favors subsequent bacterial decomposition of muscle. Gibbons and Reed (21) studied the effects of autolysis on the subsequent bacterial attack in the muscle and kidney tissues of haddock. They found that the degree of autolysis,

preceding the introduction of bacteria, did not affect the growth rate but did make a marked difference in the subsequent chemical changes.

Ripening and tendering beef by aging in coolers with controlled humidities and temperatures is the common method employed in peace times in large meat plants. During the holding period, molds of several genera appear on the cut surfaces of the meat (usually, species of *Thamnidium*, *Rhizopus*, and *Mucor*). The bacteria found on the surfaces are usually species of *Achromobacter* and *Pseudomonas*. Hundreds of examinations of "whiskery" beef stored for tendering have revealed this flora. There is a sharp difference of opinion on the question whether or not microörganisms aid in producing the organoleptic qualities demanded of aged beef or whether autolysis produces these effects.

Under ordinary conditions beef is held about 5 days at 36° to 38°F (2.2° to 3.3°C) after slaughter before it is removed to various departments for final disposition. If the beef is to be held for "ripening" so that increased tenderness and flavor develops, it is observed that at higher temperatures shorter holding periods are required. For instance, beef held at the following temperatures and times show practically the same degree of tenderness (as judged by experts):

21 days at 34°F (1.1°C)
8 days at 40°F (4.4°C)
5 days at 47°F (8.3°C)
3 days at 60°F (15.6°C)

At the expiration of these holding periods at the various temperatures the meat should be handled promptly. There is no noteworthy difference in flavor of the lean meat, of the fat, or in the juiciness of the comparable steaks.

The prevention of shrink, preservation of bloom, and control of microörganisms on meats are subjects on which experiments go on continuously. The student is referred to the work of A. W. Ewell (15–18) for the practical effects of ultraviolet light and ozone on beef and foods in refrigerators of high humidity.

Many investigations have been made on the tendering effect of enzymes on sausage casings, ground meats, and other protein foods. Many enzymes have been used experimentally for this purpose. The enzyme of the osage orange (macin) is a strong protease, as is the asclepian from the juice of the milkweed. The action of the protease of edible mushrooms has long been observed when frying meats with fresh edible mushrooms. Ficin, the rapid acting protease of figs, can be used; and bromelin, the strong proteolytic enzyme in fresh pineapple juice, has been used for some time in tendering casings of frankfurters (80). Papain, the strong proteolytic enzyme of papaya, has been used in the Americas for a long time, Kilmer (52); and the proteolytic enzyme of the *Aspergillus oryzae-flavus* group can be used for any tenderizing of foods. None of these preparations can be used in inspected plants without permission of the Federal Meat Inspection Division.

The temperature at which such an enzyme becomes inactivated is very important; the ideal preparations are those inactivated at about 170F (77C). If the

enzyme is not inactivated at 165 to 175F (74 to 79.5C) during culinary heating the meat tends to become mushy or butyrous in texture which is organoleptically undesirable.

Enzymes are not used in tenderizing hams or in the preparation of ready-to-eat hams; to accomplish this end, the ready-to-eat hams are cooked in the smokehouse, and tenderized hams are heated in the smokehouse to an inside temperature of 137F (58.4C) or higher.

3. ACTION OF MICROÖRGANISMS ON FATS

The action of microörganisms on oils and fats (other than butter fat) is a field awaiting extended study. Bacterial and mold metabolism of proteins and their derivatives and of carbohydrates have been studied extensively with brilliant results; but in the case of fat metabolism greater difficulties are encountered both in the few varieties of microörganisms that will grow on such substrates and the character of the split products. Again, many of the mechanisms of oil and fat "spoilage" have not generally been considered by oil chemists to be due to microbial action. To be sure, pure refined fats and oils freed from tissues and moisture are more prone to attack from atmospheric oxygen, light, and active catalysts like copper or iron than to direct contact with subsequent growth of microörganisms; but it has been proved in large-scale tests that fats, produced from animal and vegetable tissues that have been handled under the best sanitary conditions, show greater stability than fats processed from tissues showing large numbers of lipolytic and oxidizing microörganisms.

Colin H. Lea, who has done extensive work on this subject, states (59): "It is only comparatively recently that oxidizing enzymes have come to be regarded as potential accelerators of oxidative rancidity in foodstuffs. The earliest work appears to have been that of Jensen and Grettie (44), who inoculated fats with oxidase-producing organisms and found that the development of rancidity was frequently accelerated. The method they used for identifying oxidase-formers was to plate out the mixed organisms onto fat-emulsion agar and, after incubation, to flood the surface of the medium either with dimethyl-p-phenylenediamine, or with the Nadi reagent. Colonies of oxidase-producing organisms treated in this way stained red or violet-blue, owing to oxidation of the reagent to a highly colored quinonoid compound. More recently similar colour reactions have been obtained from the soya bean and from pork adipose tissue, indicating that these too contain enzymic oxidizing systems. Such colour tests, however, only afford evidence of general oxidizing conditions, since hydrogen peroxide produced as a by-product of any enzymic oxidation would, in the presence of peroxidase, oxidize p-phenylenediamine and the Nadi reagent. It is, of course, quite possible that fat also might be oxidized by nascent hydrogen peroxide under these conditions.

"There seems little doubt, however, that certain types of micro-organisms do accelerate the production of oxidative rancidity in fats. Cases have been observed, for example, where beef fat stored under conditions favourable to the

growth of micro-organisms has rapidly lost its induction period (determined after extraction from the tissue), though separate experiment had indicated that the activity of the tissue oxidase was probably too low to account for this result."

Thoroughly dry, pure fats are incapable of supporting bacterial growth. However, when fats contain over 0.3% moisture it has been observed that micro-organisms can grow on them. The constituents of fats other than glycerides may be important in rancidity. Jensen and Grettie (45) state: "Oils and fats from natural sources contain besides fatty glycerides small amounts of other materials, such as high molecular weight alcohols, hydrocarbons, protein residues and other nitrogenous matter, phosphatides and carotenoid pigments, which cannot be completely removed by any degree of refinement. Many of these minor constituents enter into and affect the reactions contributing to spoilage regardless of the source of the oil or fat. Their presence tends to confuse the study of the spoilage of oils and fats because they not only may decompose to small amounts of odoriferous substances but may act as catalysts, bacterial substrates, or inhibitors either retarding or promoting reactions contributing to the spoilage of oils and fats."

These writers conclude that microörganisms may cause (1) oxidative rancidity, (2) hydrolysis, (3) tallowiness, and (4) flavor changes owing to the production of various volatile products. It appears, therefore, that microbially induced rancidity is more extensive than has been realized.

The literature in this field, and the present status of the problems may be found in C. H. Lea's "Rancidity in Edible Fats," 1938 (58), H. K. Dean's "Utilization of Fats," 1938 (14), and L. B. Jensen's "Microbiology of Meats," 1942 (40).

4. EFFECT OF NITRATES AND NITRITES ON BACTERIA IN CURED MEATS

(*a*) *Action of Nitrates.* In the field of the microbiology of meats, there are few subjects over which there has been more controversy than on the effect of sodium nitrate on anaerobic bacteria in meats. Nitrate has for many decades been used in limited amounts together with sodium chloride to "cure" meats, both in pieces and comminuted. Nitrate is reduced by microörganisms in part to nitrite which forms by chemical action the heat-stable, nitric-oxide derivatives of the meat pigment, myohemoglobin. Since 1925, the Bureau of Animal Industry (now Meat Inspection Division) has permitted the use of sodium nitrite *per se* (not over 200 ppm) in meat-curing mixtures. It has been found, however, that nitrate is needed in the curing mixture and that there is much merit in the use of both nitrates and nitrites.

In 1907 Richardson (83) pointed out that "the most beneficial effect of nitrate in the curing of meat is its transformation of what would, otherwise, be *anaerobic conditions*, into aerobic ones in the bacteriologic sense. . . . I need only say here that it has been shown that aerobic bacteria will develop in the absence of air if nitrate is present in the culture medium and the anaerobes refuse to grow in the absence of air and presence of nitrate. The fact that typical putrefaction (Fäulnis) is an anaerobic process and that this process can be transformed into an

aerobic one by the presence of saltpeter, is a most important point in the curing of meat."

Grindley, MacNeal, and Kerr (23), in an extensive research on the effect of nitrate on bacteria in curing meats, found that in acid solutions (meats are usually at pH 5.9 to 6.1) even small amounts of nitrate exerted a marked inhibitory effect on bacteria in the curing vat. The growth of salt-tolerant bacteria was restricted by small amounts of nitrate in the pickling brine.

Summarizing his own work, Tanner (94) wrote: "While meat packers desired a nitrate cure a few years ago, results of extensive investigations indicate that, at times, a mixed cure (containing both sodium nitrate and sodium nitrite) is desirable from the standpoint of both spoilage and development of toxin by *Clostridium botulinum*. The preserving effects of such curing solutions have been generally attributed to the nitrites, either those added as such or those secured by reduction of the nitrate; however, evidence has been recently collected which might indicate that the nitrate itself may have some value as a preservative. This position is suggested by the fact that curing solutions containing nitrate have shown preservative action even though the nitrate reducers have been destroyed by the process."

Large-scale tests were performed in the Swift & Company Research Laboratories (36) on the effect of sodium nitrate[1] on spiced ham containing approximately 10,000 spores of *Clostridium sporogenes* per gram in 6-pound cans. The cans, and lots without nitrate added, were then processed for 5 hours at 68.3C (155F) according to a schedule no longer in use. The experiments showed that nitrate is of benefit in preventing the growth of *Clostridium sporogenes*. It was also observed that spores of some species of *Clostridium* are killed at lower temperatures and at shorter holding periods when heated in the presence of 0.1% $NaNO_3$. This adjuvant effect of nitrate was investigated by Yesair and Cameron (114) who found that the effect of curing salts on the thermal death time of spores of the test organism is apparently to reduce the time at temperatures lower than 230 to 235F (110 to 113C), and the slopes of the thermal death-time curves are greater when the salts are added. This phase of the subject is deserving of further study. When spores of *Clostridium botulinum* are incorporated in cured meat and thermal death-times compared with those of the spores incorporated in uncured meat, the inhibitive effects of the curing salts are apparent, but when the heated meat is subcultured in a liquid medium the spores are shown to be viable. Resistance values determined by subculture after heating are approximately the same in the cured and uncured meats. One of the shortcomings of nitrite is that it reacts with protein when heated at canning temperatures and is destroyed, and thus if nitrate is not present the meat in cans might not be well protected from germination of resistant spores of clostridia.

The sensitivity of species of *Clostridium* to oxygen still awaits satisfactory explanation (91). It is held by some bacteriologists that in the absence of catalase much H_2O_2 can be formed by many bacteria or accumulates, and that the amount

[1] To each 200 lbs. meat were added 4 oz. (0.125 %) $NaNO_3$, 0.5 oz. (0.015 %) $NaNO_2$, 3.5% NaCl, 2% sugar, spices.

of peroxide formed might kill vegetative forms of sporing anaerobes or retard their development. This view has been objected to on the grounds that prolonged aeration of culture media containing material oxidizable by these bacteria does not form H_2O_2. Also when catalase is added, certain clostridia do not grow aerobically. Again, it is held that washed suspensions of *Clostridium sporogenes* aerated in an oxidizable medium fail to take up oxygen.

Leifson (60) found, however, in extended experiments on spore-forming bacteria, that only vegetative anaerobic cells were oxygen sensitive. These cells were so sensitive that most of them were killed by less than 2 to 3 minutes of exposure to air. The change from the nonsensitive spore to the exceedingly sensitive vegetative cell was so abrupt that only in a few cases was the sensitivity of the intermediate stages obtained. His results also show the well-known effect of nitrate on the germination of bacterial spores. He found 1% sodium nitrate to inhibit sporulation of *Clostridium botulinum.*

McLeod and Gordon (72) obtained their evidence of peroxide formation by observing greening of heated blood agar (chocolate agar) a few millimeters below the surface at a zone where penetrating atmospheric oxygen meets with growth of clostridia. It is possible that the greening action is due to production of thiol compounds which may, on contact with oxygen, form H_2O_2. On the other hand, H_2S may form sulfhemoglobin or related compounds which, upon contact with oxygen, change into the green compounds described in Section 1.

When *Clostridium botulinum* is grown under anaerobic conditions on the surface of blood-agar plates containing benzidine and then exposed to air, sufficient peroxide accumulates to produce dark halos. This test shows peroxide production in the presence of oxygen. Hence, since strict anaerobes apparently do not produce catalase, the organisms are unable to destroy the toxic oxidizing compound. (Peroxidase does not decompose H_2O_2 in the absence of an oxidizable substance, whereas catalase decomposes H_2O_2 in the absence of an oxygen acceptor.)

The work of Hart and Anderson (26) indicates that the green discoloration produced on chocolate agar can be reproduced anaerobically by the action of reducing systems, including cysteine and bacterial suspensions with hydrogen donators. The identity of the green pigment is not clear, but the above evidence is taken by many writers to prove that hydrogen peroxide is not the only "reagent" which can form a green zone in blood pigments. Hart and Anderson state that discoloration of heated blood agar (chocolate agar) by certain streptococci is inhibited by catalase which is direct evidence that the greening is due to H_2O_2. The greening action on unheated blood media by the pneumococci and streptococci is viewed by them as an entirely different phenomenon. The discussion in Section 1 may extend some of these views.

The reader is referred to the work of Broh-Kahn and Mirsky (9) who do not agree with McLeod and Gordon's peroxide theory. They believe also that the reduction potential theory of Quastel and others is equally untenable. It may be concluded that no theory at the present time explains why anaerobes cannot grow in air, and in the writer's opinion, meat technologists should reserve judg-

ment as to the effect of nitrates on clostridia and the mechanisms of green-pigment formation until this field is more fully developed.

The important work of Tarr (98) on the action of nitrite on bacteria, recently reported, needs serious attention in evaluating the mechanisms of growth retardation of anaerobes and aerobes in mixed cure with nitrate.

(*b*) *Action of Nitrites.* Tarr (98) has shown that nitrites may, under certain conditions, play an important part in retarding the growth of many kinds of bacteria and thus delay the spoilage of meats. The work done by other investigators has indicated that large amounts of nitrite—greatly in excess of 0.02% nitrite permitted by law—are necessary to kill or inhibit bacteria. Since previous workers did not state the pH of the media they employed, it may well be that the media were neutral or faintly alkaline. Tarr found that bacteria are susceptible to low concentrations of nitrite only at pH values below 7. Not all bacteria studied proved equally susceptible to nitrite, and certain organisms proved to be resistant. The growth of species of the following genera at pH 5.7 to 6.0 was either inhibited or prevented by 0.02% of sodium nitrite: *Achromobacter*, *Flavobacterium*, *Pseudomonas*, *Micrococcus*, *Escherichia*, *Aerobacter*, and one species of *Torula*. Tarr found that in the acid range 0.02% $NaNO_2$ also inhibited the growth of two species of obligate anaerobes, *Clostridium botulinum* and *Clostridium sporogenes*. Likewise, 0.02% $NaNO_2$ inhibited the growth of *Eberthella typhosa* and *Staphylococcus aureus*. While Tarr does not attempt to explain the mechanisms of inhibition, he points out that nitrites may combine with respiratory hematin compounds. Ingram (34) found that oxygen uptake in the case of the aerobic, cytochrome-containing *Bacillus cereus* is inhibited by traces of nitrite. There is little doubt that nitrite acts directly upon the bacterial cell, but whether its action is general or specific is not clear, according to Tarr. He found that $NaNO_2$ in concentrations of 1 and 10% exerted marked bactericidal action in acid medium, but not above pH 7. In this connection, Bittenbender *et al.* (5) reported that *Staphylococcus aureus* was not killed upon exposure to 38.8% of sodium nitrite for 10 minutes at pH values between 3 and 8. These workers did not report the numbers of organisms inhibited but recorded only the presence or absence of growth.

Quastel and Wooldridge's (79) work suggests that nitrites inactivate certain enzymes by combining with their amino groups.

The theory that inhibition of the growth of sporing anaerobes during meat-curing processes is due to H_2O_2, a substance which is toxic for clostridia even in extremely small amounts, and which may accumulate through inhibition of catalase by the hydroxylamine formed in cures (46), may well account for some growth inhibition; but Tarr's phenomenon shows that nitrites also retard clostridia under such conditions. Some recent work has demonstrated that hydrogen peroxide does possess antibacterial action. Green and Pauli (22) have observed that the antibacterial action of the xanthine oxidase system is due to the formation of H_2O_2, a product of the enzymic action. Van Bruggen, Raistrick, *et al.* (105) have shown that penicillin B (an enzyme of flavoprotein nature) owes its bactericidal powers to H_2O_2, a product of the enzymic action. McCulloch

(70) writes that H_2O_2 can prevent the growth of anaerobic organisms although its influence is transitory, but it is incapable of destroying the spores of anaerobes.

When nitrite in meat is heated at canning temperatures above 100C (212F), much of the compound is destroyed. Brooks, Haines, Moran, and Pace (10) have observed that at 212F the time required for fifty per cent destruction of nitrite increases with the initial concentration ranging from 13 to 120 minutes for values of 30 to 589 g $NaNO_2$ per 10^6 g tissue. With the usual times of cooking, the reduction from a high to a low nitrite content cannot be expected.

The presence of nitrate in a finished sausage or other mixed-cure product on occasion presents the disadvantage of continued production of nitrite by bacteria during transit to some inspection laboratory or during improper storage in the chemists' locker before undergoing analysis. Much misunderstanding has arisen from this avoidable bacterial action, and cured meat foods showing over 200 ppm of nitrite upon ultimate analysis have undoubtedly often left the sausage kitchen with the lawful limit of nitrite present. It is not clear how the nitrate-reducing bacteria can grow in the presence of such large amounts of nitrite found in experimentally incubated, mixed-cure sausage.

It is possible that the characteristic flavor of bacon and ham (as opposed to salt pork) is due to a product of a reaction of nitrite with constituents of the tissue, either during curing or during cooking, according to Brooks, Haines, Moran and Pace (10).

(*c*) *Action of Nitrates on Gas Formation by Species of Bacillus.* The typical gaseous swell of canned cured meats is practically always due to the fermentation of sugar by species of the genus *Bacillus*. Large quantities of carbon dioxide are formed when either the small or large varieties (except Donker's group) grow in a medium containing fermentable carbohydrates, nitrates, and cured pork meat, or in "sugar-cured meats" at temperatures ranging from 23.9 to 48.9C (75 to 120F). This gaseous fermentation also produces spongy beef hams and causes bursts in large bologna. None of the hundreds of strains examined can form gas in the ordinary sugar broths or sugar-agar media.

Nitrate, sugar, and cured meat must be present together before the bacilli ferment with gas production. If the medium contains c.p. nitrite without nitrate, no carbon dioxide forms. If sugar is omitted, no carbon dioxide, or very little, is formed. If cured meat is omitted and nitrate, sucrose, and any ordinary soluble proteins or peptones are added, no carbon dioxide forms. With some strains of the bacilli, gas is formed if nitric-oxide hemoglobin or nitric-oxide hemochromogen is present in the sucrose-nitrate medium. Some strains of the genus *Bacillus* do not form acid in the standard sugar-veal infusion broths (47) but do form acid and carbon dioxide when grown in nitrate sugar-cured pork medium. This was observed with the disaccharides and one of the pentoses, xylose. In the case of sucrose-nitrate-cured spiced ham carbon dioxide is formed as follows: The first reaction observed is the splitting of some of the disaccharides to monosaccharides. Later, lactic acid is found in appreciable quantities and also much carbon dioxide. In the closed container, as used for canned meats, where there is no escape for acetaldehyde, much 2,3-butylene glycol is formed. The order ap-

pears to be disaccharide to hexoses to triose compounds and then to pyruvic acid. A part of the pyruvic acid is reduced to lactic acid and a part is split into acetaldehyde and carbon dioxide. The acetaldehyde may either be reduced to alcohol, oxidized to acetic acid, or condensed to form acetyl methyl carbinol which can be reduced to 2,3-butylene glycol.

We have never observed the formation of carbon dioxide in media other than nitrate-sugar-cured pork meat or nitric-oxide myohemoglobin or hemochromogen except in a few instances where thiamin was added to the nitrate-sugar medium. Presumably, phosphorylated vitamin B_1 acts as a coenzyme which decarboxylates pyruvic acid; and there is a comparatively large amount of thiamin in pork. Thiamin is not a complete factor in this mechanism since gas was not observed to be formed by many strains of bacilli capable of gaseous fermentation in the nitrate-sucrose-cured pork medium (47).

The spores of this group are killed by the present-day processing schedule for canned spiced ham or luncheon meats. In the presence of 3 to 3.5% NaCl, 2.33 oz (0.15%) $NaNO_3$, and 0.125 oz (0.008%) $NaNO_2$ per 100 pounds of meat, the 6-pound cans may be inoculated with spores of clostridia, bacilli, or thermophiles and retorted at 112.8C (235F) for 3.5 hours so that an inside temperature of 107.2C (225F) is reached with a finished product that will keep at temperatures below 55.5C (132F).

ZoBell (116) has demonstrated the ability of some microörganisms to destroy nitrites as they are produced from nitrates, and recommends that tests for nitrates be made in conjunction with tests for nitrites when the latter substance is not found. In this connection, we have observed that hydroxylamine may be found in the old-style, long-mixed cure for hams towards the end of the curing period (60 to 80 days). The present-day ham is cured in less than 20 days. Lindsey and Rhines (68) have demonstrated the production of hydroxylamine from the reduction of nitrates and nitrites by various bacteria. Hydroxylamine hydrochloride, when added to nutrient agar, inhibits the growth of food-poisoning varieties of staphylococci in dilutions of 1:20,000, and many of the common molds in dilutions of 1:15,000. Kitasato (53) has reported that hydroxylamine when added to culture media inhibited the growth of *Clostridium tetani*, *Clostridium chauvei*, and *Clostridium septicum*.

5. ACTION OF SALT (NaCl) ON BACTERIA

(*a*) *Action of Salt on Bacteria in Meats.* For the past half century the demand for heavily salted foods has lessened in many parts of the world. Salt-preserved fish and meat were for a long time staple articles of food in world trade, and the preservation of foods by salting has proved its efficacy since time immemorial (55). The New England-West Indies trade in salted meat was of importance in colonial times (99).

Lessening the amounts of salt used in food preservation has resulted in changes in microbiological flora and brought forth many phenomena of interest to the bacteriologist. Tanner and Evans (95) have discussed the literature on the effect of salt on pathogenic bacteria and stated that most investigators indicate that

salt in the concentrations used in food preservation is not a bactericide but acts as a preservative by inhibiting many species of bacteria. Tanner and Evans conclude from their own extensive experiments that salt is the most active preservative used in meat-curing solutions.

Petterson (78) has shown that the preserving action of salt in meat and fish is not sharply defined. Salt exhibited a selective action and did not inhibit all bacteria. Anaerobes were inhibited by 5% NaCl whereas aerobic rods, facultative anaerobic rods, and micrococci were not greatly inhibited. The rod forms were more easily suppressed by salt than the cocci. Ten per cent NaCl appeared to inhibit most bacteria, although a few grew in media containing up to 15% NaCl. Various yeasts appeared to persist and grow in media containing over 15% NaCl.

At this point attention is called to the diverse results which can be observed when, for instance, 5% salt is used in pickling meats or is added to liquid culture media. Tanner and Evans (95) have demonstrated that the inhibitory effect of salt on *Clostridium botulinum* is different when the same concentrations of NaCl are used in the different culture media. Glucose agar containing 6.5% or more of NaCl did not support growth. Nutrient broth containing 6.7% NaCl allowed one culture to show visible signs of growth and five strains of *Clostridium botulinum* to grow and produce toxin. It was necessary to add 7.8% NaCl to prevent the growth of all strains in nutrient broth. When 7.3% NaCl was used in glucose broth and 7.8% in pork-infusion broth, growth and toxin formation took place. Growth took place in 10.5% NaCl in glucose broth and 12% was needed to inhibit growth in this medium. With cooked pork toxin formation was prevented by 5% NaCl.

When over 3.5% NaCl is used in curing whole pieces and comminuted meats, most anaerobes are suppressed although there are a few strains of nonpathogenic, sporogenous anaerobes that can germinate and multiply when given heat treatment several times greater than that necessary to destroy spores of *Clostridium botulinum*. Fortunately these strains are encountered very infrequently and at this time are a curiosity in culture collections.

Bacillus foedans of Klein, which we have equated with *Bacillus putidus* of Weinberg (48), is inhibited in hams containing 3% NaCl and undergoing the smokehouse processing. This organism is very proteolytic and grows in nonpickled areas, sometimes formed through by-passing the bifurcation of the iliac artery during pumping. This condition may be differentiated from sarcosporidial activity, which likewise causes a butyrous area, but is associated with a normal salt content and normal organoleptic characteristics.

Many species of aerobes and facultative anaerobes (as well as strict anaerobes) can grow in high salt concentrations in brines which contain large pieces of various animal tissues; but the growth takes place at the interfaces of brine and tissues and not readily in the clear brine. When meat infusions or whole blood are added to 15 to 20% clear NaCl brines, the growth of salt-tolerant microörganisms is very slow. When large pieces of meat are added to these vats and the salometer readings are kept constant, growth proceeds more rapidly, usually on the surfaces of

the meat. The mechanisms of growth at the interfaces appear not to be well understood at the present time.

In the curing of comminuted meats, the ratio of salt to total moisture obviously accounts for the low quantities of salt which suffice to inhibit many microorganisms. For instance, in composite beef muscle the water content is about 71% and protein 18.7% with a water-to-protein ratio of 3.8, while in lean pork the water content is 64.5% and protein 18.9% with a water-to-protein ratio of 3.4 (74). A higher ratio of salt to water accounts for the preservative effect of salt in meats as compared to liquid culture media; but the problem of free and bound water in tissues confronts one here as in so many other fields of food technology. Long experience has taught the curing departments of the industry the precise amounts of salt necessary (in addition to the lawful amounts of nitrates and nitrites) to be effective in each of the many kinds of meat foods.

(*b*) *Effect of Impurities in Salt.* A phase of the salt curing of meat which has received attention from time to time is the effect of impurities in the salt. Many commercial meat processors are of the opinion that impurities in common salt (calcium, magnesium) affect the meat adversely, whereas some believe that more desirable cures, from the standpoint of rapid penetration, are affected. Moulton and Lewis (75) made up sodium chloride curing solutions containing 5% $CaCl_2$, or 1% $CaCl_2$ + 1% $MgSO_4$, or 1.5% $CaCl_2$ + 0.5% $MgSO_4$ for curing hams, butts, and beef knuckles. They found no consistent effects of these impurities in sodium chloride brines upon the rate of penetration of the NaCl into the meat. They concluded that commercial salts would show no measurable differences in penetration rates owing to the usual impurities.

There is an extensive literature on the physiologically antagonistic effects of ions (20). There is evidence that the toxic action of an ion can be nullified by another different ion. In the case of bacteria there is a quantitative antagonism but the qualitative effects of all cations may be alike. The toxic effect of a monovalent salt like NaCl can be neutralized by the addition of a divalent salt in suitable proportion. Winslow and Falk (112) have found that 0.145 M $CaCl_2$ + 0.290 M NaCl was toxic to *Escherichia coli*, but a solution of 0.145 M $CaCl_2$ + 0.680 M NaCl was nontoxic.

Tressler and Murray (102) have noted that the presence of calcium and magnesium compounds as impurities in salt accelerated the development of the salt-fishy odor and taste of salted fish. It has been observed that if pure salt was used for the salting of fish, the product when thoroughly freshened was nearly indistinguishable from fresh fish. Tressler (101) had observed that impurities in the salt exerted a depressing action on the permeability of the cell walls and thus slowed down the penetration of salt into the muscle tissues. The various effects of salt impurities have been studied by E. Hess (28) who states: "Spoilage, as measured by rise in trimethylamine and bacterial content, of cod press-juice containing 21 grams salt per 100 ml. (about 80% saturated) is delayed longest with pure NaCl, and increasingly less with mined evaporated, mixed crude, Mediterranean and Turk's Island solar salts. This order corresponds to decreasing percentages of NaCl and increasing percentages of impurities in these salts. The

differences in the salt action increase with lower temperatures." Hess used fish-muscle press-juice instead of the muscle itself which eliminates the penetration factor and also makes bacterial counts more accurate. Hess quotes unpublished data of Gibbons who observed that a medium not containing calcium or magnesium ions (prepared from dialyzed drip of fish muscle, washed agar, and pure NaCl) did not support the growth of red halophilic bacteria, or only very poorly, whereas the addition of traces of calcium or magnesium or replacement of the sodium chloride by solar salt resulted in good growth. The same effects can be observed on cells of red halophilic bacteria in the presence of $CaCl_2$, $MgCl_2$, and $MgSO_4$ in high concentrations of NaCl. Beef cured in 100° salometer brine with solar sea-salts and stored at 32.2 to 37.8C (90 to 100F) will spoil with characteristic trimethylamine odors after 5 months, showing a typical flora of *Serratia*, *Achromobacter*, and *Micrococcus*. The work of Tarr (97) is interesting in this connection in that he has discovered an enzyme, "triamineoxidease," existing in cells of five different genera of bacteria which can form trimethylamine.

The dry-curing process employed to cure country hams and southern-style hams is interesting from the standpoint of salt permeability. In observing this practice in many places in America, it was noted that the farmer or commercial processor first rubs sodium nitrate over the ham, usually rubbing from the shank towards the butt. The shank (tibia and fibula) is "sawed long" to effect marrow sealing. The nitrate appears to increase permeability or "cause swelling and a moist condition." Then after a time salt or salt and sugar are rubbed into the ham in the same manner as the nitrate. The process is repeated a number of times for about seven weeks, and then the hams are smoked for varying lengths of time. The effect of nitrate on the permeability has not been given much scientific study (110), but the art of curing in this manner is very old. Surprisingly few pieces cured in this way show frank spoilage (as distinguished from incipient "souring").

It is known that very dilute nitric acid softens the connective tissue binding the muscle fibers. This action may aid in "tendering" certain cured meats. Studies are needed on the action of nitrates, nitrites, salt, sugar, and enzymes on muscle fibers, sarcolemma, sarcoplasm, myofibrils, discs, and other structures of muscle.

Rockwell and Ebertz (86) conclude that the preserving effect of NaCl involves more than its dehydrating capacity. For instance, if common salt inhibits bacterial growth by means of dehydration, then other equally efficient dehydrating salts should serve as inhibitors. Magnesium sulphate has greater dehydrating effect on proteins than NaCl but is not as efficient in preventing the growth of *Staphylococcus aureus*. They conclude from their experiments that the preserving action of NaCl on proteins involves more than dehydration, there being four other factors operative. These factors are: the direct effect of the chloride ion, removal of oxygen from the medium, sensitization of the test organism to CO_2, and interference with the rapid action of proteolytic enzymes.

(*c*) *Bacteria in Salt.* From time to time, salt contaminations leading to spoilage have been suspected in meat processing, but no unanimity of opinion exists either among food bacteriologists or operating men in the industry concern-

ing the hazards of contamination in salt. There are even those food bacteriologists who doubt the existence of obligate halophiles as spoilage organisms, and who view these bacteria as salt-tolerant forms. The distinction is not clearly made in some of the literature on salt. The observations of Clayton and Gibbs (13), Robertson (85), and ZoBell, Anderson, and Smith (117) indicate that halophilic bacteria are distinct species indigenous to environments containing high concentrations of sodium chloride. ZoBell *et al.* (117) found an average of 167 obligate halophiles in Great Salt Lake water having a salinity of 27.6%. These halophiles required at least 13% salt for growth. These bacteria are believed to have become acclimatized to increasing salt concentrations during the time that the water of old Lake Bonneville evaporated to leave its saline remnant, Great Salt Lake.

The studies of Stuart, Frey, and James (92) and Stuart and Swenson (93) indicate that halophilic bacteria are adapted forms of ordinary bacteria known to be indigenous to other environments, such as soil, etc. Lochhead's work (69) on halophilic bacteria should be consulted in this connection.

The data obtained during the past decade (37) from microbiological analyses of mined salt and vacuum-pan salt of various grades indicate strongly the improbability of contaminations in salt sufficiently important to lead to meat spoilage. Certain of the solar sea-salts may, however, need careful scrutiny. Yesair (113), who examined many grades of salt, advised microbiological examinations of the salt as a control measure in the food industry.

The question of the nature and origin of halophiles has been reviewed elsewhere (37).

(*d*) *Some Effects of Salt on Bacteria.* Large initial numbers of contaminating bacteria tend to grow in meats that are highly salted. The observation has been made many times that large numbers of non-halophilic or non-salt-tolerant bacteria tend to neutralize the inhibitory effect of salt. It appears that the larger the inoculation the greater the salt tolerance of the bacteria. The work of Sherman and Albus (89) shows that when active reproduction takes place there is marked destruction of the cells in the presence of 5% NaCl. This observation is borne out in practical application where it is noted that fresh meats showing light bacterial contamination keep better when salted quickly than when held for a time before salting. The old cells are not as sensitive to salt as the actively growing cells.

The effect of sodium chloride on thermal death times of bacteria has been studied by Esty and Meyer (19) who found that NaCl in low concentrations (0.5 to 1%) markedly increased the thermal resistance of spores of *Clostridium botulinum.* At 2% concentration of salt, this effect was lost. Up to 8%, little or no effect of the salt was observed. Above this concentration, up to 20% NaCl solution, the effect was to decrease the thermal death times. Viljoen (106) studied the adjuvant effect of NaCl upon thermal death times of spores in pea liquor. He found a protective influence of salt in the range of 1 to 2.5% NaCl, and 3% salt shows about the same protection as 0.5%. Four per cent NaCl increases very slightly the destructive effect of heat.

In the case of micrococci, salt appears to exert a protective action against the effects of heat. Alkaline solutions of salt reduce the thermal death times of spores in a very striking manner.

The terms "obligate halophile," "salt tolerant," "salt resistant," and "facultative halophile" may all be employed to indicate the characteristics of a strain, but there is still difference of opinion as to the distinction between such terms. If Kluyver and Bahrs' (54) doctrine of "physiological artifacts" is true, i.e., that halophilism is developed by conditions of artificial culture in saline media from a prepotency latent in the cell of the saltless environment, much remains to be done before the terminology becomes clear. They demonstrated the interconvertibility of nonhalophilic *Microspirum desulphuricans* (Beijerinck) and halophilic *Microspirum aestuarii* (Van Delden) by gradual alteration of the salt content of the medium. They suggested that these forms are all derived from a common parent strain, and hence the specific identity of the halophiles and non-halophiles. F. B. Smith has written an excellent review of this subject (90).

There are many strains of obligate halophilic molds, usually divergent forms of brown molds, that exhibit all degrees of salt tolerance. Some are psychrophiles, but most strains are mesophiles. Some resemble species of *Torula*, others resemble species of *Hemispora*, *Oospora*, or *Sporendonema*. *Aspergillus candidus* will grow on salted smoked meats and produce small reddish patches.

6. SOME RECENT DEVELOPMENTS IN MEAT TECHNOLOGY

The war has brought about some interesting developments in meat technology as well as in food technology generally. The outstanding developments in the production of foods on a scale hitherto undreamed of have been along the lines of dehydration of pork, beef, eggs, milk, soups, and some poultry meats. Boneless, well-trimmed beef (produced under bacteriological control) and other meats are frozen; and through the efficient handling of the Quartermaster Corps and Veterinary Corps, these meats are made available for consumption in battle zones. The microbiology of freezing meats and of all kinds of stored fresh and cured meats and frozen eggs has taken on new life. Bacteriological studies have shown that there are critical storage temperatures at which the "indigenous" flora die off rapidly. For instance, we (42) have shown that frozen egg magma inoculated with several strains of *Escherichia coli*, several species of *Pseudomonas*, *Achromobacter*, *Flavobacterium*, and *Micrococcus*, when frozen, divided into two lots, and then held at −22 F (−30 C) and 22 F, (−5.6 C) shows sharp differences in reduction of all bacteria in four weeks. Strangely enough, the bacteria are reduced to very low numbers of viable cells at 22 F; and there is very little or no diminution of viable cells to be found at −22 F. There are also critical holding temperatures for beef, pork, lamb, veal, and dressed poultry. Atmospheric oxidations complicate some of the benefits derived from the "higher" temperatures of storage.

(*a*) *Dehydration of Meat.* Processes for dehydration of meat of all kinds have come to the fore during the war years, and the reader is referred to the excellent work of von Loesecke (108) for details of the drying and dehydration of foods. Four kinds of meat have been prepared: fresh meat cooked and raw, and cured

meat cooked and raw. Most of the dehydrated beef and pork of commerce is precooked fresh meat dried to 10% moisture and then packed and sealed in tin cans. Extensive studies carried out by the U. S. Department of Agriculture and the meat packers have shown that from a bacteriological standpoint dehydrated meat, made in accordance with Federal specifications, is safe and will remain so when stored without refrigeration in hermetically sealed containers (57). In such dehydrated meat, we notice a rapid decline in the numbers of bacteria. The pH is 5.8 to 6.2. *Salmonella sp.* staphylococci, *Clostridium botulinum*, and other pathogens will not grow in this product until the meat is rehydrated to contain over 30% moisture.

Commercial desiccation of foods is no new thing. During the Civil War troops were supplied with desiccated vegetables, soup mixtures, apples, peaches, etc. General Sherman wrote in his memoirs: "During the Atlanta campaign we were supplied by our Commissaries with all sorts of patent compounds, such as desiccated vegetables, and concentrated milk, meat biscuits and sausages, but somehow the men preferred the simpler and more familiar forms of food, and usually styled these 'desecrated' vegetables and 'consecrated milk'." Dehydrated rations were used in the Boer War; and the A. E. F. veterans of 1917, who accounted for the consumption of 10 million pounds of desiccated foods, exhibited about the same attitude as their grandfathers towards these dried foods. Following World War I the dehydration of foods was not extensive, but with the coming of World War II this business has taken on new life. Improved methods may save the industry from complete collapse after the war ends.

An interesting application of rapid dehydration of meat has been employed by Ritchell, Piret, and Halvorson (84) to form products like dry or summer sausage. They investigated air-drying of *uncooked cured* meats. In contrast to the usual sausage drying operations, shorter times of drying were necessary to obtain a product containing 25 to 30% moisture.

Work done by Dr. E. E. Rice and Dr. H. E. Robinson of the Research Laboratories of Swift & Company shows that:

Protein quality is not significantly reduced during dehydration or canning unless diets very low in proteins are considered.

Vitamin retention of pork and beef undergoing dehydration or canning is similar to that for household cooking of similar meats, being thiamin, 60 to 70%; riboflavin, 90 to 100%; niacin, 90 to 100%; and pantothenic acid, 70 to 80%.

Cured pork undergoing commercial canning (12-oz. can) retains 67 per cent of its thiamin, 90 per cent of its riboflavin, 94 per cent of its niacin, and 76 per cent of its pantothenic acid. Thiamin retention in 6-lb. cans is lower, being 40 to 50%.

During storage of either canned pork, dehydrated pork or dehydrated beef at temperatures up to 99 F (37.2 C) there is little or no loss of niacin, riboflavin, or pantothenic acid over a period of 219 days. Above 120 F (48.9 C) there are slow losses of riboflavin and pantothenic acid. Thiamin decreases more rapidly, showing some loss at 80 F (26.7 C). After 293 days' storage the thiamin retention in canned pork is 52 per cent. In dehydrated pork the retention is poorer, being 28 per cent after 219 days at 80 F (26.7 C). At higher temperatures there is almost complete destruction of thiamin in both products.

Molds can develop on dehydrated meats when these foods are exposed to the atmosphere and stored at relative humidities below 75% in the temperature range

of 10 to 37.2 C (50 to 99 F). Molding is, however, not a hazard in the tins and other containers used in distribution of this product.

(*b*) *Bacterial Standards.* The bacteriological control of foods by the criteria of agar-plate counts has long been a "standard method" for the inspector and producer. Most bacteriologists have been confronted with the dilemma of evaluating a food product from the "count" or the more laborious examination.

The subject of bacterial counts, i.e., bacteria growing into colonies in agar plates when incubated in the range 20 to 37 C (68 to 98.6 F) for varying lengths of time, as criteria for judgment of many foodstuffs is controversial at the present time. One school of thought would throw over the existing agar-plate count standards for most foods and replace this method by qualitative bacteriological examinations together with organoleptic criteria and chemical examinations. This group claims that when, let us say, a food like dried eggs contains 500,000 "viable aerobes" per gram and the "standard limits" are 250,000 per gram, the egg powder should not be rejected as inedible. There is undoubtedly much to be said for this position. However, the regulatory laboratories must have some criteria and limits of numbers if their investigators are to rely on "standards of numbers of bacteria permissible." The total number of bacteria growing on agar plates do, in many instances, serve to guide the processing of foods so that incipient spoilage does not result.

In meat foods, for the most part, it is more important to know both the kinds and numbers of the flora present than to know merely the numbers of microorganisms. Because most bacteria which grow in meat under refrigeration are psychrophiles or facultative psychrophiles, such as many species of *Achromobacter*, *Pseudomonas*, or *Serratia*, it may be considered that a "high count" does not necessarily indicate that the food is unsound if the organoleptic characteristics are satisfactory. Again, if counts are made on certain sausages, such as Thuringer, the number of lactobacilli found is very great indeed (40).

In the preparation of frankfurters, bologna, meat loaves, and other comminuted meat foods, the bacteriologist must be guided by qualitative bacteriological data. The total agar-plate count cannot presage the possibility of oxidizing effects leading to discolorations.

Meat, when produced under Federal inspection, is not likely to harbor pathogens, according to the data of the Bureau of Animal Industry, the Meat Inspection Division, or the Research Laboratories of the Meat Industry. There are no "normal" plate counts for meats in the literature available to us. A satisfactory count on meats may depend upon whether or not the meat is destined for use in sausage or, in the case of beef, whether or not it is to be aged in coolers for some time to induce tenderness, flavor, and juiciness. As there are several hundred kinds of meat foods produced continuously, no simple answer is forthcoming in respect to a "single standard" for these foods. It would be helpful if we could state with conviction founded upon experience that liver sausage should not exceed sixty thousand aerobes per gram or that pork links or patties should not exceed several hundred thousand aerobes per gram. However, perfectly edible sausage might show three times these figures, depending upon the length of time the food has been stored in the shop or commissary refrigerators.

It would appear that the ideal laboratory examinations of a finished food product should disclose the presence or absence of pathogens or toxins, and that both plate counts and determination of flora may be used in control of the processing. Methods for direct microscopical counts of foodstuffs are not wanting in number. Some inherent difficulties in direct-count methods are: (a) numbers less than 1×10^5 organisms per gram in food substances, such as muscle, cannot be accurately counted; (b) dead cells are counted (this is not a serious error); (c) errors in sampling, preparation, grinding, smearing, etc., are not eliminated; (d) "primitive" forms are not detected (41), nor are types determined except in a very general manner. Other shortcomings are perhaps apparent to the analyst, but in theory the method of direct count obviously has much to commend it. However, the problem is not considered impossible of solution, and the direct count could supplant the agar-plate count in many places.

(*c*) *Hams.* New applications of old methods of establishing bacteriological criteria for the curing and smoking of hams (not tenderized, not ready-to-eat) have been effected and incorporated into official specifications. These applications are based on the bacteriological observations showing that cured hams should be smoked at temperatures below 125 to 130 F (51.7 to 54.4 C) for several days for the smoke to penetrate well, that some dehydration should occur, and above all, that the tissues do not become denatured through heat coagulation. It is well known to the bacteriologist that coagulated proteins, such as cooked meats or boiled eggs, are more vulnerable to enzyme action, and bacteria find a more suitable substrate for growth on them than on raw substances. Hams and shoulders, when tenderized (partial cooking), or cooked in the smokehouse so that they are ready to eat, are to be considered in the same category as comminuted, cured domestic sausage products which must always be kept under adequate refrigeration. It has been shown (43) that cooked hams, cooked poultry and stuffings, sandwiches prepared with ham, eggs, fish, and fowl, and soup stocks should never be held in the "incubation zone" of staphylococci longer than four hours if gastrointestinal irritation due to staphylococci is to be prevented. The incubation zone is considered to be from 60 to 115 F (15.6 to 46.1 C), and as a safety margin for institutional and military mess halls the range from 50 to 120 F (10 to 52.9 C) is advocated.

(*d*) *Control of Spores in Canned Meats.* The heating schedules for canned meats developed through the stimulus of war have been based largely upon existing data derived from studies of spores. The direction of the work in the practical canning of spiced ham, luncheon meats, and sausage products is to retort the canned food according to a "botulinum schedule." Many meat foods subjected to this temperature would not be very palatable; hence, the need arose to produce a canned meat that would neither spoil nor present a health hazard. Also high-temperature processing for long periods lessens the vitamin content of meat foods. The thiamin and pantothenic acid content, especially, may be reduced, although niacin and riboflavin are not significantly reduced. When 3.5% NaCl, 2.33 oz. (0.15%) $NaNO_3$, and 0.125 oz (0.008%) $NaNO_2$ in each 100 pounds of comminuted meat are used, and the cured meat is packed in 6-pound cans and retorted for 3.3 hours so that the inside temperature reaches 225 F

(107.2 C), the processed cans of meat, even though seeded with spores of *Clostridium sporogenes* and of *Bacillus sp.*, will keep at any temperature below 132 F (55.6 C) for a time depending upon the rate of production of hydrogen from the chemical action of the contents upon the metallic can (hydrogen swells).

As much as 22% soya flour or "meat extender" can be added to comminuted meats and canned according to this schedule of curing and heating with the resulting product stable under expected temperatures (132F [55.6 C] and lower). Tests were conducted by Jensen and Hess (49, 50) who inoculated soya flour to contain over one million spores of gas-forming, anaerobic thermophiles per gram. Thirty 6-pound cans of comminuted luncheon meat (pork and beef) containing 22% of this soya flour were incubated for 9 months at 132 and 99 F (55.6 and 37.2 C) and found to be stable. The control cans without salt and nitrate swelled and burst in a few days at these incubation temperatures.

The "bombage" of canned meat foods stored without refrigeration in the tropics has been caused by viable spores of *Bacillus sp.* These spores are not inhibited from germination by 3.5% NaCl with sodium nitrate. These spores must be killed to produce a stable canned pork or luncheon meat. Spores of thermophiles may remain dormant in canned meat for long periods of time. Recently, Wilson and Shipp (111) examined bacteriologically a can of roasted veal, packed for the explorer Parry in 1824, and recovered six strains of sporing bacilli. The strains grew best at 37 C, all strains grew well at 55 C, and some grew at 60 C. The survival of spores in this can of veal in viable condition for over a century appears to have no parallel in bacteriological records.

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