A STUDY OF THE BACTERIOLOGICAL CHANGES PRODUCED DURING THE AGING OF CURED HAMS.

by

Daisy I. Purdy

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I

INTRODUCTION.

The long aging of dry-sugar-cured hams is a customary practice in the South, especially in Maryland, Virginia,and Kentucky. Connoisseurs of old hams seem to think that from one to two years are required to develop the particular flavor, aroma, and texture which is most desirable. During this aging process there is a great loss in the weight of the hams. Often times in a group of hams cured at the same time, under apparently the same conditions, not all will age properly. Some hams will be a complete loss because of spoilage due to improper cure, mold contamination, or other undetermined causes; others will fail to develop the desired flavor or aroma, or both, thus producing an undesirable product.

In earlier days when meat was preserved for family use only, many took this loss from spoilage and lack of proper aging as a matter of course, but today, it has become quite an economic problem. Accordingly, the project, "The Effect of Aging on Cured Hams" was

undertaken at the University of Maryland Agricultural Experiment Station as a cooperative project, involving three departments of the University of Maryland (Departments of Animal Husbandry, of Chemistry, and of Bacteriology) and the Bureau of Animal Industry of the U. S. Department of Agriculture. It was hoped that this study would determine the changes produced in the hams during the aging process, and, also, some of the factors responsible for producing these changes. This information would help to insure a uniform cure and probably the length of time required to produce the desired flavor, aroma, and texture could be reduced, thus affecting considerable saving. In any case, when the proper conditions are known, loss by spoilage should be materially reduced. This study of the bacteriological changes produced in cured hams during the aging process is, therefore, only one phase of the project under investigation.

II

HISTORICAL.

A search of the literature fails to show any report of work carried out to determine the kind and extent of bacterial flora present in hams after they had been out of cure for varying length of time. Practically all of the bacteriological work done with hams has been in connection with the study of ham souring. McBryde (1911) conducted the first scientific study of ham souring and concluded that it was caused by an anaerobic bacillus which he named Bacillus putrifaciens.

Boyer (1923, 1925) isolated and identified from fresh chilled hams McBryde's Bacillus putrifaciens and four other anaerobes. From the sour bone marrow he consistently isolated two species of anaerobes which he believed to be the cause of spoilage. These latter two were also present in the fresh chilled hams. He found spoilage always started at the center of the largest muscle of the carcass. He concluded that if bacteria are present in the fresh hams, the necessity for thorough chilling, as quickly as possible after slaughter, is highly desirable as the first step in producing good

hams. A. F. Reith, working under the Arthur Lowenstein Fellowship at the University of Chicago, made a study of the bacterial flora of the muscular tissue of live hogs, of fresh normal hams taken from hogs on the killing floor, and of hams at various stages during the curing process. He came to the same conclusion as Boyer. Reith (1926) in a report of this work gave the following data.

Table I.

Studies on Miscellaneous Hams.

Point at which taken	No. of Hams Examined	No. of Samples	No. Showing Growth	No. Showing Anaerobic Growth
From hams of live hogs	6	36	36 (100%)	20 (56%)
After sticking and before scalding	8	39	36 (92%)	20 (51%)
After scalding and before dehairing	8	39	31 (79%)	20 (51%)
Immediately after dehairing	8	41	28 (68%)	22 (54%)
Immediately after eviscerating	11	52	43 (83%)	38 (73%)
After 48-hour chill	4	20	16 (80%)	14 (70%)
After 5 days in cure	1	5	4)	2)
After 10 days in cure	1	5	5) (87%)	4) (60%)
After 20 days in cure	1	5	4)	3)

He grouped the bacteria which he isolated from the tissues and blood of apparently normal hogs as follows:

Aerobic cultures (94 cultures were classified morphologically)

59 per cent staphylococci

22 per cent bipolar rods

19 per cent Gram-positive rods

16 per cent coccus forms other than staphylococci

6 per cent Gram-negative

Anaerobic cultures (59 cultures classified on the basis of their cultural reactions)

19 per cent strongly proteolytic

40 per cent feebly proteolytic

10 per cent non-proteolytic

30 per cent no growth or uncertain reactions.

Sturges (1923) in studying the flora of meat curing solutions, found that a highly complex flora developed after a few days; he isolated and studied the following:

(1) A yeast (torula)

(2) A motile Gram-negative bacillus resembling B. coli in morphology

(3) A very pleomorphic form

(4) A bacillus (?) which persistently curves

(5) A bacillus which invariably develops long filaments bearing peculiar convoluted nodules at frequent intervals

(6) A vibrio resembling cholera vibrio in morphology

(7) A spherical form - Gram-negative and extremely motile, which was a typical salt tolerant organism of the flora he isolated. It was a nitrate reducing, non-sporing form, developing readily in concentrationsof NaCl up to 15 per cent. It did not produce gas nor liquify gelatin.

The work of Reith shows that there is very little difference in the per cent of samples showing growth, irrespective of the stage of the curing process from which the sample is taken. Hess (1928) studying the bactericidal action of smoke as used in smoke-curing of fish, found that the resistance of bacteria to smoke was greatest in media of optimum salt concentration. He used artificial media instead of fish in his experiments because conditions could be better controlled. He found non-spore formers were killed in 1 to 2 hours under conditions of the experiments; spores were very resistant, resistance increasing with age of spore cultures. The smoke penetrated very irregularly through fish tissue, penetration increasing with the concentration of the smoke. The influence of proteins (peptone solutions and fish extracts) upon the bactericidial action of smoke was to "quench" it; this was most noticeable in less dense smoke. Bactericidal

action of smoke in buffered media was greatest in acid medium, decreasing toward the alkaline side of neutrality.

Much work has been done on the use of nitrates and nitrites in the curing of meats. McBryde (1911) showed that KNO_3 had no more preservative power than common salt against organisms of spoilage. Haldane (1901) and Hoagland (1910, 1914) have shown that the function of nitrates in the curing process is the fixation of color which takes place thru the reduction of nitrates to nitrites and thru the combination of the latter with the hemoglobin of the meat to form NO-hemoglobin. Haldane ascribes this function to bacteria.

Kerr, Marsh, Shcroeder and Boyer (1926) note the fact that the variability of the cure in new establishments can be overcome by adjustment and control of the P_H of the pickle and by seeding the pickle with a vigorous, effective NO_3-reducing strain of bacteria. They found considerable variability in the vigor of growth and rapidity and completeness of the conversion of nitrates to nitrites exhibited by various strains isolated. Herein may lie one of the secrets of the successful curing and aging of the famous dry-

sugar-cured hams of the South where the same curing, smoking, and storage rooms have been used for generations. These rooms are probably seeded, not only with a vigorous, effective NO_3-reducing strain of organism, but also, with other organisms which aid in producing the desirable flavor and aroma in the hams during the aging process, as has been found to be true in the case of different cheeses.

Supplee(1931) found from chemical analyses that during the aging process the per cent of free fatty acids greatly increased. The meat-fat, i.e., the inter-and intra-muscular fat, contained a higher per cent of free fatty acids than the external fatty layer.

Falk, Noyes, Sugiura(1925) studying lipase action of extracts of the whole rat at different ages found progressive change with age. The absolute lipase action reached a maximum at times varying between 124-40 days for the 10 different esters they used. Roma and Losnitzki(1926) in investigating the effect of lipase of various fresh tissue on tributyrin showed the importance of finely disintegrating the sample for comparative measurements. Willstater, Haurowitz and Memmer(1924) point out that with gastric lipase the

purer the lipase the more the optimum P_H changes from 5.5 to 7.9. The lipase extract was unstable in alkali but stable in weak acid.

The fact has been conclusively shown that tissues of apparently normal animals are not sterile and also, that the salt and smoke concentration used in the curing of meats do not kill all the organisms present. Therefore, it is interesting to note in connection with the problems under investigation that a number of micro-organisms have already been reported which produce lipase. Eijkman(1903) demonstrated lipase formation in cultures of Staph. aureus, B. pyocyaneus, B. prodigiosus, B. flourescens liquefaciens and non-liquefaciens. Waksman and Davison(1926) state that "lipase is also produced by B. cholerae, B. typhosus, B. indicans, B. ruber, Staph. pyogenes albus, Strep. hemolyticus(work by Thompson and Meleney (1924)), B.lipolyticus, Micr. tetragenes, bacterial spores(work by Ruehle,1923) and B. tuberculosis(work by Wells and Corper, 1912)." Thus it would seem plausable that some of the lipase production in the tissues might be due to organisms present, as well as being a function of the tissues themselves. Corran(1929) found a low concentration of NaCl and KCl in water slightly

augmented the action of lipase, also, a low concentration of lecithin and of cholesterol in water did the same.

III

EXPERIMENTAL.

A. Methods

1. Methods of Study

After the isolation of organisms from the samples of ham (as described elsewhere), the 1929 Descriptive Chart of the Society of American Bacteriologists was used to record the characteristics of the organisms studied. The methods included in the "Manual of Methods for the Pure Culture Study of Bacteria", prepared by the Committee on Bacteriological Technic of the Society of American Bacteriologists were followed in so far as possible. The organisms isolated anaerobically were tested for aerobic growth. All cultures were run thru sucrose, dextrose, lactose and nitrate broths and, also, litmus milk. Gram stains (using the Hucker Modification) were made of all the cultures and most of them were tested for gelatin liquifaction. Nitrate reduction was tested by means of the sulphanilic acid-a-naphthylamine test as recommended in the "Manual of Methods". The lack of time prevented making further

differentiation and classification of the organisms isolated. The work with molds was started, using the method of Thom (1910, 1926, 1930), but was not completed because of the time factor involved.

Preliminary tests were run on the effect of varying salt concentrations on the growth of organisms; also on the effect of varying concentrations of agar. For these tests the basic pork-infusion media (described elsewhere) was used, the desired additions being made to this.

2. Equipment

The usual laboratory equipment was used, with the exception that porous cups for Demonstration jars, size 3x7.5 cm. inside, was found to be the most satisfactory containers for the phosphorous which was used in providing anaerobic conditions for the growth of cultures in the museum jars.

3. Sterilization of Equipment

The most satisfactory method found for sterilizing the glass mortars and pestles used, was to add the sand to the mortar and then place it and the pestle in a tin culture dish holder (size 3 inches by 4 7/16 inches, a type

frequently used for sterilizing petri dishes). If the pestle would not go into the holder, it was wrapped separately in paper. The scissors and forceps used were wrapped in cheese cloth and then in paper, for sterilization. Petri dishes, pipettes, and solution bottles, as well as the above apparatus, were sterilized in the hot air sterilizer for 1 hour at 180°C.

4. Anaerobic Seals

For sealing the anaerobic plates, a mixture of 9 parts of paraffin to 1 part of vaseline was placed in an 8 inch porcelain evaporating dish. A convenient working height was obtained by placing this dish upon a tripod. The edge of the anaerobic plates was rotated in the melted paraffin-vaseline mixture, keeping the edge of the plates near the side of the evaporating dish rather than in the middle during the rotation. If there was a considerable difference in the diameter of the two plates used, filling the space between the two plates with the sealing mixture by means of a teaspoon, and allowing it to cool, facilitated the operation. A neat, smooth, even seal on the plates was obtained by the above procedure; also, the time required for sealing the plates was shortened very much, at the same time giving a more satisfactory seal than was possible by any other method tried.

Museum jars were evacuated as follows; a thin layer of cotton was placed in the bottom of the museum jar, after which the inside of the jar was lined with paper toweling. This aided in keeping the moisture produced in the jar from collecting later on the sides of the test tubes and causing the identification numbers to rub off of the test tubes. The inoculated tubes were placed in the jar and the toweling was brought over the top of the tubes and placed down in the center of the jar, making a place for the porous cup containing the phosphorus. A tube of methylene blue solution, as suggested by Hall (1921) was placed in each jar to test for the absence of oxygen. After adjusting the cup in the center of the jar and applying a mixture of 9 parts of vaseline to 1 part of paraffin to the ground glass rim of both jar and lid and to both sides of the rubber gasket, a stick of red phosphorus (1 inch long by 5/8 inch in diameter) was placed in the porous cup. When the phosphorus ignited the lid was put in place and the seal effected by means of an adjustable clamp. The majority of the museum jars had a slight imperfection where the rim had been molded on. It was found that this small air passage could be satisfactorily sealed before filling the jar, by applying to the lower side of the rim, a mixture of 9 parts paraffin

to 1 part vaselin, such as was used to seal the anaerobic plates.

The shake-agar culture tubes were sealed with a six to ten centimeter layer of 3 per cent washed agar, which was poured into the test tube after the inoculated nutrient agar had solidified.

5. Media

The basic media for all isolation work was pork infusion media containing 1 per cent Bacto-peptone, 0.5 per cent NaCl, and 1 per cent glucose, as suggested by McBryde (1911), except that in the present study it was found to be more practical to substitute agar for broth; hence, 1.8 per cent agar was found expedient because, in securing the 1 to 6.25 dilution, it was necessary to use 2 cc. of the 1 to 12.5 dilution per plate.

Rettger's (1906) "egg-meat mixture", as modified by McBryde (1911) and used with such marked success in growth of putrifactive organisms, was tried, but growth was isolated so seldom fromit that its use was discontinued. Media as suggested in the "Manual of Methods for the Pure Culture Study of Bacteria" was used for all differential media. All media was adjusted to neutrality with Brom-thymol-blue as the indicator and was auto-claved at 15

pounds pressure for 30 minutes. For the growth of molds, Thom's (1910) formula originally adapted from Czapek by Dox(1910) was used.

B. Procedure

1. Sources of Hams

The cultures reported upon in this investigation were isolated from hams from various sources. These hams had been subjected to different curing processes and were of different ages, i.e., they had been held for various lengths of time after having received their last smoke.

Part of the hams used in this study were from three groups of hogs raised, slaughtered, and cured under the supervision of the Department of Animal Husbandry of the University of Maryland in connection with the project "Effect of Aging upon Cured Hams". According to the plan of work for the project, these hogs were treated as follows: one lot of each group was full-fed in order to gain as rapidly as possible; the other lot was fed a limited ration and reached approximately the same final weight as the full-fed lot in about six months more time. All of the above hogs were fed until an average weight of approximately 300 pounds was attained. The concentrates of the ration in both lots was composed of corn and fishmeal. The hogs were on pasture in summer and in the winter were given alfalfa hay.

The hogs were slaughtered and the hams were cured at the U. S. Animal Husbandry Experimental Farm,

Beltsville, Maryland. A dry cure was used which consisted of 8 pounds of salt, 2 pounds of granulated sugar, and 3 ounces of salt-peter to each 100 pounds of green meat. One half of this mixture was applied when the meat was first put down; one fourth, three days later and the remainder was applied when the pack was fifteen days old. The hams were cured at a temperature between 36° and 38°F. The curing time was two days per pound of green meat. Following the curing time, the hams were withdrawn from the pack and were hung up, unwashed, and allowed to air cure for two weeks. After this period the hams were washed, allowed to dry, and were smoked for two hours per day on alternate days until they had received four smokes. The temperature of the smoke was kept below 100°F. The hams were wrapped in parchment paper, placed in a muslin bag, and were stored in a dry, dark, well ventilated room at the Maryland Agricultural Experiment Station. This cure will be designated in this study as the "standard"cure".

2. Hams used for Analysis

The following "standard cure" hams were used

for bacteriological examination:

Two hams just out of cure (not raised experimentally but given the "standard cure");

One full-fed ham which was aged for four months;

One slow-fed, eight months ham;

Two- slow-fed, 12 months hams;

One full-fed, 12 months ham;

Two slow-fed, 24 months hams;

One full-fed, 24 months ham.

The series was not complete because the feeding experiments were discontinued.

In addition the following hams were also examined:

Three Swift Premium hams, just out of cure;

Three Joyner's Smithfield, six months to one year old;

Two hams from Chestertown, Maryland, 12 months old, designated as Nicholson hams;

Two hams from Howard County, Maryland, 12 months old, designated as Wolfe hams;

Two hams from Prince Georges County, Maryland, one 12 months old and the other 36 months old, designated as Crandall hams.

The Maryland farm-cured hams were cured by a dry-sugar-cure process similar to the "standard cure" with the exception of the "Crandall" hams. These latter hams were

brine-cured, approximately the same curing mixture being used as in the dry-cured, but the mixture was made into a brine instead of being applied dry to the hams.

3. Sampling of hams

In any hams where the cut surface was contaminated with mold, loop specimens of characteristic types present were transferred to slants of Dox (1910) agar for later identification. After removing any specimens of molds desired, the excess mold was removed by means of a damp cloth. The hams were sectioned through the body, being cut an inch to an inch and one-half below the aitch bone. (Figure 1 shows a cross section of a ham thus cut.) The samples required for bacteriological examination were removed aseptically from the shank end of the ham, after which the hams were used for physical and chemical examination. (These two latter examinations are being reported by others.)

Before removing the samples from the interior of the hams, the surface of the ham over the area to be sampled was seared by means of a platinum spatula. A plug of the desired size, 3 to 8 grams, was removed by means of sterile, sharp-pointed scissors and forceps,

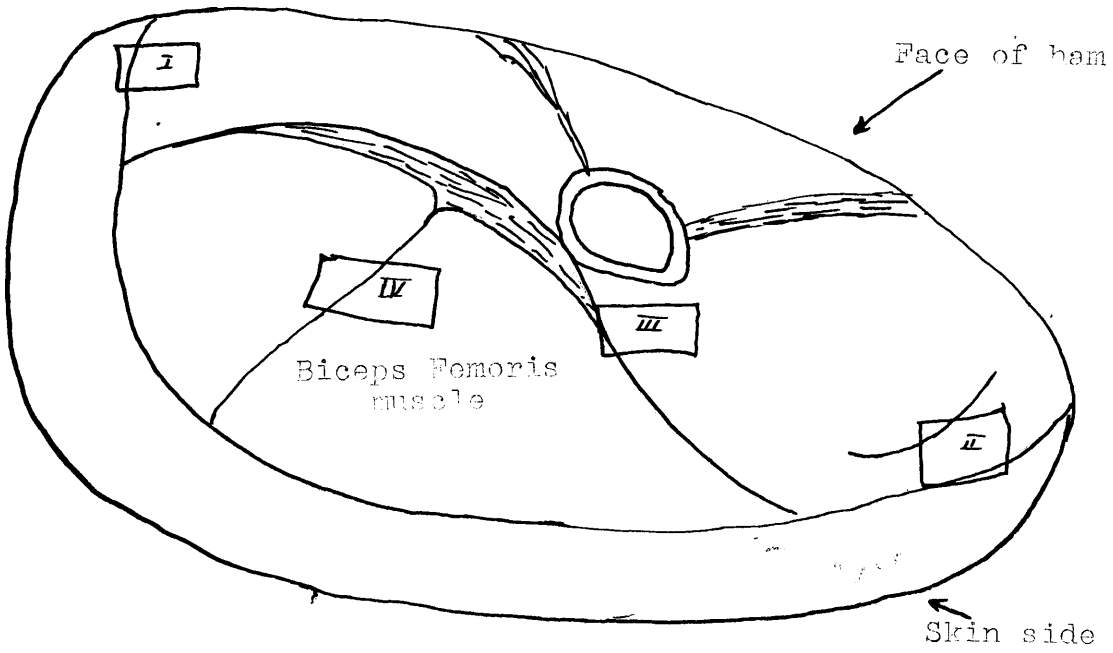

FIGURE 1. Cross-section of ham showing the positions from which samples for analyses were taken.

and was placed in a sterile petri dish to be removed later for maceration. The petri dishes were counter balanced on a Harvard Trip balance and the size of the plug removed was determined by weighing.

The samples were taken from approximately the same position in each ham (see Figure 1). Samples 1 and 2 always contained a portion of the external layer of fat; sample 3 was always taken as near as possible to the bone, on the bottom side, i.e., from the side nearest the external fat layer or skin side; sample 4 was taken from about the middle of the lean surface, including at least a part of the large triangular muscle, the biceps femoris muscle. Before taking a second series of samples (numbered 5, 6, and 7) from the same ham, a slice about two and one half inches thick was cut from the ham in order to remove the surface from which the previous samples had been taken. Sample 5 corresponded in position to 1; sample 6, to 2; and sample 7, as a rule, corresponded approximately to sample 4. Any variation from the above is indicated in the individual experiment.

4. Maceration and Dilution of Sample

The maceration of the samples and the pouring of the agar plates were carried out under a glass hood. For

maceration of the sample a sterile mortar and pestle and sterile sand were used. It was found that the maceration could be greatly facilitated, especially in the case of the larger samples, if the sample was first cut rather fine, by means of sterile scissors, directly into the mortar. The proportion of approximately 1.5 grams of sand per gram of ham to be macerated, was found to be quite satisfactory. After the sample of meat was well macerated with the sand, it was made up to a dilution of 1 to 12.5, using sterile water. The water was added to the mortar in thirds. It was found that most of the sand-meat mixture could be transferred to the sterile solution bottle with the first third. The remaining sterile water was used to remove to the solution bottle as completely as possible, the sand-meat mixture which adhered to the mortar and pestle. The bottle was then closed by means of the sterile cap, and the contents were shaken vigorously, by hand, for one minute, giving approximately 100 shakes per minute. After allowing the mixture to settle for about 5 minutes, samples were removed for bacteriological analysis.

5. Isolation of Cultures

Anaerobic plates were poured in triplicate,

using the method of Krumwèide and Pratt (1913) and earlier, of Marino (1907), but with the modification of seal on the plates as described elsewhere. Aerobic plates were poured in triplicate. Dilutions of 1 to 6.25 (made by using 2 cc. of the original 1 to 12.5 dilution of sample per plate) and 1 to 50 were used for both types of plates. Shake agar cultures, with a seal of 3 per cent washed agar, were made in duplicate, using dilutions of 1 to 12.5 and 1 to 50. All cultures were incubated at room temperature for ten to fourteen days, after which the different types of colonies were picked from each and placed on agar slants. The anaerobic slants were placed in a museum jar which was evacuated by means of phosphorus. (Sellards, 1904)

Lipase Test

In order to ascertain whether or not a measurable amount of lipase was extracted from the ham sample during maceration, the Kanitz(1905) method as modified by Rosenheim and Shaw-Mackenzie(1910) was used. For these tests highly refined cotton-seed oil was secured from a commercial company who submitted the following approximate composition of the oil: linolein, 55 per cent; stearin, 10 per cent; olein, 35 per cent.

Tests on each sample of ham were run in triplicate, as follows: 10 cc. of cotton-seed oil was placed in a sterile 125 cc. Erlenmeyer flask to which was added 2 cc. of 1 to 12.5 dilution of macerated meat in water (the same sample as was used in pouring the plates) and 1 cc. of toluol. The oil was practically neutral to phenolphthalein, and preliminary tests gave approximately the same results with the oil as purchased as after neutralization which accords with the findings of Willstätter et al(1924); therefore, the majority of the tests were run with the unneutralized oil. Triplicate controls were run with each test; the control flasks differed from the test flasks only in that the enzyme preparation was boiled to kill the enzyme. At first the preparation was boiled over a Bunsen flame for one minute, but results obtained seem to indicate that not all the enzymatic activity had been destroyed; hence, steaming for one hour in live steam was adopted. Both test and control flasks were incubated at 37°C. for 96 hours, as this was found to be the time required for equilibrium to be reached. At the end of the incubation period, 50 cc. of 95 per cent acid-free alcohol, 5 cc. of acid-free ether, and 3 drops of 1 per cent phenolphthalein in 50 per cent alcohol

were added to each flask. Tenth normal NaOH was used to titrate the free-fatty acids. The average of the amount needed for the three controls was then subtracted from the average required for the three tests in order to obtain the amount of tenth normal NaOH required to neutralize the free-fatty acids formed by the lipase. By using the formula suggested by Lewkowitsch (1921), the number of cubic centimeters of N/10 NaOH required was converted into per cent oleic acid. The formula follows:

$$\frac{\text{cc. of N/10 NaOH} \times 0.282}{\text{cc. of oil} \times \text{sp.gr. of oil}} \times 100 = \text{per cent oleic acid}$$

In the present work the normality of the alkali used was 0.1166, or it contained 0.0046 grams NaOH per cc. Therefore, the factor to be multiplied by the number of cubic centimeters of N/10 NaOh used, was found to be:

$$\frac{0.0046 \times 0.282}{0.040 \times 10 \times 0.9203} \times 100 = 0.352 \text{ per cent}$$

This is considering the molecular weight of oleic acid to be 282. Multiplying 0.352 by the number of cubic centimeters of N/10 NaOH gives the per cent of oleic acid formed by a dilution of 1 to 6.25; to reduce this to the basis of a 1 gram sample, the value obtained above would need to be multiplied by 6.25.

SERIES I.

The object of this series of experiments was to determine the total count of organisms, aerobic and anaerobic, per gram of sample from hams which were just out of cure, i.e., they had just been given their last smoke; also, to group these organisms morphologically, in so far as time permitted.

The hams used in this series were: - (1) Two hams taken from hogs not experimentally fed, but given the "standard cure". (The use of these particular hams was made necessary because of an accidental loss of two hams just out of cure from experimentally fed hogs.) The above hams were used for analysis on the day after they had received their last smoke. The color of the skin of these hams was light brown; there was present but a very small amount of surface salt. Examination of the freshly cut surface showed the fat to be soft in texture and white to reddish white in color; the lean was very soft and moist and was unevenly colored, ranging from pale pink on the interior near the bone, to dark red near the upper cut surface. The outside had a moderately pronounced smoky odor. The aroma of the freshly cut surface was that of a

fresh cured ham, suggestive of sweet fresh pork. Ham V weighed 13.6 pounds, while ham W weighed 15.2 pounds.

The second group in this series were Swift Premium hams, Swift & Company's standard cured product. They were taken immediately from the smoke room of the plant and brought to the laboratory for analysis. The color of the skin was a pale golden brown. There was no surface salt. The freshly cut lean surface in ham No. 2 SP was a fairly uniform pink; the fat was soft and reddish white in color. The lean of ham No. ISP was spotted, varying from light red to grayish pink and of ham No. 3SP was "firey" red around the bone, getting paler towards the surface. In all three of these hams the lean was very soft and moist; the aroma of smokiness was pronounced on the outside; for the cut surface, the aroma of fresh cured ham was slightly pronounced, the sweet, fresh pork odor predominating. The hams varied in weight from 13.1 pounds to 14.7 pounds.

Table II. gives the bacterial counts per gram for aerobic and anaerobic plates poured in triplicate, and for shake agar cultures made in duplicate.

TABLE II.

Bacterial Count per gram for hams from different sources just after smoking.

(Plate counts in triplicate, shake agar culture counts in duplicate)

Sample No. **	Beltsville Ham * Series No. V.			Beltsville Ham * Series No. W.			Swift Premium Series No.1 SP			Swift Premium Series No.2 SP			Swift Premium Series No.3 SP		
	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture
I (1)	94	44	-	6	-	12	-	-	-	100	100	125	-	-	25
(2)	56	12	25	-	6	12	-	-	25	50	50	-	-	-	25
(3)	18	44	-	12	-		-	25		100	100		-	-	
II (1)	500	36	600	6	-	-	-	25	50	75	125	175	Uncountable		
(2)	500	18	800	-	-	-	-	25	-	150	25	200	"		
(3)	585	18		12	12		25	-		75	350		"		
III (1)	18	18	-	120	12	12	Uncountable			-	50	-	-	50	50
(2)	18	-	25	70	lost	37	"	"	"	25	50	-	25	25	75
(3)	12	12		110	18		"	"	"	-	-		-	25	
IV (1)	Uncountable			56	6	37	150	225		525	350	600	-	-	-
(2)	"	"	" "	36	-	25	250	150	275	525	350	650	25	25	25
(3)	"	"	"	18	-		250	275		550	300		-	25	-
V (1)	18	18	50	12	-	25									
(2)	100	36	85	56	30	12									
(3)	50	25		50	-										
VI (1)		-	25	-	-	-									
(2)	lost	-	12	18	-	-									
(3)		-		-	-										
V II (1)	-	-	-	56	-	37									
(2)	-	6	-	44	12	25									
(3)	6	-		60	-										

* Beltsville hams given the "standard cure"

** I, II, etc. represent different samples, taken as described on p.

Very few of the plates which showed growth had many colonies on them. The organisms isolated from the three plates which contained numerous pin-point sized colonies, were not materially different from the organisms isolated from the other samples.

Table III classifies the organisms from Series I according to their Index Numbers. All the organisms isolated anaerobically proved to be facultative anaerobes. They fall into the same classification groups, with the exception of their oxygen relationships, as do the aerobic organisms. Of the organisms isolated from this series 86.0 per cent of them fell in the Micrococci group; 37.0 per cent as aerobes and 49 per cent of the total as facultative anaerobes. There was no gas produced by any of these micrococci and their acid production may be summarized as follows:

Organisms producing acid in	Aerobes		Facultative Anaerobes	
	Number of Cultures	% of aerobes	Number of culture	% of F. Anaerobes
1. Glucose	18	36	14	22
2. Glucose and sucrose	23	48	35	55
3. Glucose, sucrose and lactose	6	12	9	14

Name of organism..... Habitat..... Studied by..... Culture No.....

Date of isolation..... History..... Optimum conditions: Media..... Temp.....

MORPHOLOGY

Underscore required terms.

SKETCHES

VEGETATIVE CELLS, Medium used.....
reaction.....temp.....age.....days.
Form, *spheres, short rods, long rods, filaments, commas, short spirals, long spirals, curved.*
Arrangement, *single, pairs, chains, fours, cluster cubical packets.*
Limits of length.....; of diameter.....
Size of Majority.....
Ends, *rounded, truncate, concave, tapering*.....
CAPSULES, present on.....
How stained .....

SPORANGIA, *present, absent.* Medium used.....
reaction.....temp.....age.....days.
Form, *elliptical, short rods, spindled, clavate, drumsticks.*
Limits of length.....; of diameter.....
Size of Majority.....
ENDOSPORES, *present, absent.*
Method of examination, in ***stained*** **or** ***unstained*** preparations.
If stained, by what technic?.....
.....
Location of Endospores, *central, excentric, subterminal terminal.*
Form, *spherical, ellipsoid, cylindrical*
Limits of Size.....
Size of Majority.....
Wall, *thick, thin.*
Sporangium wall, *adherent, not adherent.*

MOTILITY
In broth..... On agar.....
FLAGELLA, No. .....Attachment, *polar, bipolar, peritrichiate.* How stained.....

IRREGULAR FORMS,
Present on.....in.....days at.....°C.
Form *spindled, cuneate, filamentous, branched,* or.....

STAINING REACTIONS.
Gram: 1 day.....; 2 day.....; 3 day.....; 4 day.....;
.....day.....Technic used.....
.....
Acid fast.....
Special stains.....
.....
.....
.....

CULTURAL CHARACTERISTICS

Underscore required terms		SKETCHES	Underscore required terms.	
Agar Stroke	Growth, *scanty, moderate, abundant, none.*		**Nutrient Broth**	Surface growth, *ring, pellicle, flocculent membranous, none.*
	Form of growth, *filiform, echinulate, beaded, spreading, arborescent, rhizoid.*			Clouding, *slight, moderate, strong transient, persistent, none, fluid turbid*
Incubation Temperature	Elevation of growth, *effuse, flat, raised, convex.*		**Temperature**	Odor, *absent, decided, resembling*.....
.....°C	Lustre, *glistening, dull.*		.....°C	.....
	Surface, *smooth, contoured, rugose.*		**Age**	Sediment, *compact, flocculent, granular flaky, viscid on agitation.*
Age	Optical Characters, *opaque, translucent, opalescent, iridescent.*		.....d	Amount of sediment, *abundant, scanty none.*
.....d	Chromogenesis.....Photogenic, Fluorescent.			
	Odor, *absent, decided, resembling*			
	Consistency, *butyrous, viscid, membranous, brittle*			
	Medium, *grayed, browned, reddened, blued, greened, unchanged.*			
Gelatin Stab	Growth, *uniform, best at top, best at bottom.*		.....	
	Line of puncture, *filiform, beaded, papillate, villous, arborescent.*		**Medium (liquid)**	
Temperature	Liquefaction, *none, crateriform, infundibuliform, napiform, saccate, stratiform;* begins in.....d		**Temperature**	
.....°C	complete in.....d.		.....°C	
	Degree of liquefaction in.....days.....		**Age**	
Age	Method used.....		.....d	
.....d	.....			
	Medium, *fluorescent, browned, unchanged.*			
.....			.....	
Medium (solid)			**Medium**	
Temperature			**Temperature**	
.....°C			.....°C	
Age			**Age**	
.....d			..... d	

BRIEF CHARACTERIZATION

As each of the following characteristics is determined, indicate in proper marginal square by means of figure, as designated below. In case any of these characteristics are doubtful or have not been determined, indicate with the letters U, V, and X according to the following code:
U, undetermined.
V, variable.
X, doubtful

PRIMARY CHARACTERISTICS

Microscopic Features

Form: 1, streptococci; 2, diplococci; 3, micrococci; 4, sarcinæ; 5, rods; 6, commas; 7, spirals; 8, branched rods; 9, filamentous

Endospores: 0, absent; 1, central; 2, excentric to terminal

Flagella: 0, absent; 1, peritrichic; 2, polar

Gram stain: 0, negative; 1, positive

Miscellaneous Biochemical Reactions

Biologic relationships: 1, pathogenic for man; 2, for animals but not for man 3, for plants; 4, parasitic but not pathogenic; 5, saprophytic; 6, autotrophic

Relation to oxygen: 1, strict aerobe; 2, facultative anaerobe; 3, strict anaerobe

Gelatin liquefaction: 0, negative; 1, positive

In nitrate media: 0, neither nitrite nor gas; 1, both nitrite and gas; 2, nitrite but no gas

Chromogenesis: 1, fluorescent; 2, violet; 3, blue; 4, green; 5, yellow; 6, orange; 7, red; 8, brown; 9, pink; 0, none

Carbohydrate Reactions

Diastatic action: 0, negative; 1, positive

From glucose: 0, no acid; 1, acid and gas; 2, acid without gas

From lactose: 0, no acid; 1, acid and gas; 2, acid without gas

From sucrose: 0, no acid; 1, acid and gas; 2, acid without gas

SECONDARY CHARACTERISTICS

Vegetative Cells

Diameter: 1, under 0.5μ; 2, between 0.5μ and 1μ 3, over 1μ

Length: 1, less than 2 diameters; 2, more than 2 diameters

Chains (4 or more cells): 0, absent; 1, present

Capsules: 0, absent; 1, present

Spores

Shape: 1, spherical; 2, ellipsoid to cylindrical

Diameter: 1, less than diameter of rod; 2, greater than diameter of rod

Cultural Features

Agar Stroke

Growth: 0, absent; 1, abundant; 2, moderate; 3, scanty

Lustre: 1, glistening; 2, dull

Surface: 1, smooth; 2, contoured; 3, rugose

Agar colonies: 1, punctiform; 2, circular (over 1 mm. diameter); 3, rhizoid; 4, filamentous; 5, curled; 6, irregular

Gelatin colonies: 1, punctiform; 2, circular (over 1 mm.); 3, irregular; 4, filamentous

Milk

Acid: 0, no acid; 1, sufficient for curdling; 2, insufficient for curdling

Rennet curd: 0, absent; 1, present

Peptonization: 0, absent; 1, present

Indole production: 0, negative; 1, positive

(Optional Spaces)

Culture No.....

Agar Colonies	Sketches: Surface Colonies	Sketches: Deep Colonies	Gelatin Colonies	Sketches: Surface Colonies	Sketches: Deep Colonies
Temperature .....°C			Temperature .....°C		
Age .....d			Age .....d		

PHYSIOLOGY

TEMPERATURE RELATIONS

Optimum temperature for growth.....°C.
Maximum temperature for growth.....°C.
Minimum temperature for growth.....°C.

RELATION TO REACTION OF MEDIUM

Medium used.....
Optimum H-ion conc., *about pH =*.. .....
Limits of pH for growth: *from*.....*to*.....

CHROMOGENESIS

Nutrient gelatin.....
Nutrient agar.....
Potato. .....
....

PRODUCTION OF INDOLE

Medium... .....
Test used.....
Indole *absent, present in*.....*days*

PRODUCTION OF HYDROGEN SULFIDE

Medium .....
Test used .....
H_2S *absent, present in*.....*days*

RELATION TO OXYGEN

Method used.....
Medium.....Temperature.....°C.
Aerobic growth: *absent, present, better than anaerobic growth.*
Anaerobic growth: *absent, occurs in presence of glucose, of lactose, of sucrose, of nitrate; better than aerobic growth.*
Additional data.....
.....
.....

MILK

Temperature.....°C.

Reaction: 1 day.....4 days.....10 days.....
Acid curd: 1 day.....4 days.....10 days.....
Rennet curd: 1 day.....4 days.....10 days.....
Peptonization: 1 day.....4 days.....10 days.....

LITMUS MILK

Temperature.....°C.

Reaction: 1 day.....4 days.....10 days.....
Acid curd: 1 day.....4 days.....10 days.....
Rennet curd: 1 day.....4 days.....10 days.....
Peptonization: 1 day.....4 days.....10 days.....
Reduction of litmus *begins in*.....*days; ends in*.....*days*

NITRATE REDUCTION

Medium.....Temperature.....°C.
Nitrite: 1 day.....2 days.....4 days.....7 days.....
Gas: 1 day.....2 days.....4 days.....7 days.....

Medium.....Temperature.....°C.
Nitrite: 1 day.....2 days.....4 days.....7 days.....
Gas: 1 day.....2 days.....4 days.....7 days.....

FERMENTATION

Temperature.....°C

Medium containing .... and:	GLUCOSE	LACTOSE	SUCROSE	XYLOSE	MANNITOL	DULCITOL	GLYCEROL	DEXTRIN	INULIN	SALICIN	STARCH				
Presence of gas in Smith tube															
Amt. of CO_2 in Eldredge tube															
First appearance of acid.....															
First appearance of alkali.....															
Reaction (pH) after.....days															
Max. H-ion conc. (in pH).....															
Carbon source consumed in....															

PATHOLOGY

ANIMAL INOCULATION

Medium used..... Age of culture..... Amount.....Incubation period.....

Type of Injection	Whole culture			Cells			Filtrate		
Animal									
Subcutaneous	*								
Intraperitoneal									
Intravenous									
Per os									

*In each instance where pathogenicity is observed, indicate location of lesion, and type, e. g. edema, histolysis, gas, hemorrhage, ulcer, diphtheritic, etc.

ANTIGENIC ACTION

Animal.....Medium used..... .....Age of culture.....
Type injection ..... Number of injections.....
Culture causes production of *cytolysins, agglutinins, precipitins, antitoxin.*
Specificity: Antibodies produced effective against other antigens as follows.....
.....
Immune sera from.....
.....effective against this organism as antigen

SPECIAL TESTS

This DESCRIPTIVE CHART prepared by the Committee on Bacteriological Technic of the SOCIETY OF AMERICAN BACTERIOLOGISTS. Presented at the annual meeting of the Society, Dec. 30, 1929.
Members of the Committee: H. J. CONN, VICTOR BURKE, BARNETT COHEN, ELIZ. F. GENUNG, IVAN HALL, W. L. KULP

TABLE III

CLASSIFICATION BY INDEX NUMBERS OF ORGANISMS ISOLATED FROM HAMS JUST OUT OF CURE

Class	Index Number	Culture Number	Total
1	30U1-U1U20-U200	W423(4)	1
2	30U1-U1020-U200	V422(1),-(2),-(3),-(4), V423(1), V424(1),-(2),-(3),-(4), V425(2)	10
3	30U1-U1120-U200	W423(3), W424(2), W424(4), W427(1),-(2),-(4),	6
4	30U1-U1020-U202	V425(3),1SP424(1),-(3),-(5)	4
5	30U1-U1120-U202	V425(1), V427, W421(1) W422(2), W423(1),-(2), W424(1),W426(3), W427(3)	9
6	30U1-U1000-U202	1SP423(1a),-(2),-(3),-(3a),-(4) 2SP424(1)	6
7	30U1-U1100-U202	W424(3),W425(1),-(2)	3
8	30U1-U1020-U220	2SP421(2)	1
9	30U1-U1020-U222	2SP421(4), 3SP424	2
10	30U1-U1120-U222	W422(1)	1
11	30U1-U1000-U222	2SP422(3),-(3a)	2
12	30U1-U1U00-U222	2SP424(2), XW421(2)	2
13	30U1(?)-U1020-U202	1SP423(1)	1

TABLE III (Continued)

Class	Index Number	Culture Number	Total
14	30U1-U2U20-U200	XV434(1),-(2),-(4), XV435(1),XV436(1), XV464(1),-(2), XV467(2),XW437(1),-(2),XW465(1)	11
15	30U1-U2020-U200	X1SP464(2),X3SP462(4),X2SP434(1)	3
16	30U1-U2U20-U202	XV432(2), XV433, XV435(2), XV461(1),-(2) XV463(2), XV464(3),XV465(1) XW434(1),XW434(2),XW463(3) XW464, XW465(2),-(3),XW467(1) X1SP431(1), X2SP464(5)	17
17	30U1-U2020-U202	X1SP434(3), X1SP464(1),-(3) X1SP434(2), X2SP461(1),-(5) X2SP463(2),X2SP464(1),-(2) -(3),-(4),-(6),-(7), X3SP464(1)	14
18	30U1-U2120-U202	X2SP461(3),-(4), X3SP431	2
19	30U1-U2U20-U220	XV461(3)	1
20	30U1-U2U20-U222	XV467(1), XW431, XW461,XW462(1), XW463(1), X1SP464(4)	6
21	30U1-U2120-U222	X2SP463(1)	1
22	30U1-U2020-U222	X1SP464(5), X2SP431(2), X2SP463(4)	3
23	30U1-U2000-U202	X2SP462(1), X3SP432(2)	2
24	30U1-U2020-U000	X2SP432(1), X3SP432(1), X3SP433(3)	3
25	30U1(?)-U2020-U202	XW433(2),XW435,XW462(2) XW463(2),-(4), XW466, X2SP462(2), X2SP463(3)	8
26	50U1-U2020-U200	X1SP463(1),-(3),-(4),-(5) X3SP462(1), X3SP432(1),-(3), XV436(2) X1SP463(2), XV432(2)	10
27	30U1-U1126-U200	W426(2)	1
28	50U1-U1020-U200	V423(2)	1

Yeast isolatedfrom 2Cr421

Because of the lack of time transfers of all of the cultures into gelatin were not made. In Table III several of the groups differ only in that their ability to liquify gelatin was not determined.

There were 8 micrococcicultures not listed above, some of which gave a definite Gram-negative reaction and others, an indefinite Gram-reaction. Of all of the cultures isolated in Series I, only 11 were rods, none of which produced spores. They formed no gas and produced acid only in glucose. Only one of the rod cultures was isolated from the aerobic plates, the other 10 were from the anaerobic plates. A few cultures of a large yeast were isolated from ham 2Cr and were not included in the counts given above.

SERIES II.

Because of discontinuation of the feeding experiments, this series contains only two hams. One of the hams was from a full-fed series of animals and was aged for four months. This was ham No. 3229, Series M. There was considerable mold on the cut or upper surface. This ham had no distinctive odor or aroma, altho it was rather suggestive of the aged hams, with a somewhat stronger odor and darker color near the top where the mold grew the heaviest. It had lost 12 per cent in weight during the four months aging, and weighed 14.4 pounds when used.

The other ham in the series, ham No. 3213, Series N, was from a slow-fed series of animals and had aged for eight months. There was considerable white mold on the surface of this ham and it had followed the connective tissue down all the way to the bone, giving the ham quite a musty-moldy odor. (This was found to be true, also, of the mate to this ham which was cooked for a palatability test). The ham had lost 18 per cent in weight during the eight months of aging and weighed when used 15.6 pounds.

Table IV. gives the bacterial counts per gram for these two hams. Table V. gives the classification by Index Numbers of the organisms isolated from them.

TABLE IV.

Bacterial Count per gram for one ham which has aged 4 months, and one 8 months.

Sample No.	Ham No. 3229, full fed 4 mo. ham Series No. M			Ham 3213, slow fed 8 mo. ham Series No. N		
	Aerobic Plates	Anaerobic Plates	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture
I (1)	100	25	125	300	75	400
(2)				200	200	700
II (1)	-	25	-	75	-	-
(2)				50	-	-
III (1)	-	75	25	125	125	200
(2)				50	25	300
IV (1)	25	25	25	25	-	-
(2)				-	-	-

The hams in Series II were used in some preliminary tests in studying the effect of varying the salt concentrations of the media on the isolation of cultures from the sample; hence, there are not as many checks as on the other hams. The above mentioned tests are being reported later.

There were only 16 organisms (with the exception of a few cultures of a large yeast from ham N,) isolated and studied morphologically in this series. They were all micrococci and 2 or 12 per cent of them were aerobes and the remaining 14 or 88 per cent were facultative anaerobes. None of these cultures produced gas and they are grouped according to their sugar reactions as follows:

Organisms producing acid in	Aerobes		Facultative Anaerobes	
	Number of Cultures	% of Aerobes	Number of Cultures	% of F. Anaerobes
1. Glucose	0	0	6	43
2. Glucose and sucrose	0	0	6	43
3. Glucose, sucrose and lactose	3	100	2	14

TABLE V

CLASSIFICATION BY INDEX NUMBERS OF ORGANISMS ISOLATED FROM HAMS AGED 4 AND 8 MONTHS RESPECTIVELY

Class	Index Number	Culture Number	Total
1	30U1-U1020-U222	N421(2), N152	2
2	30U1-U2020-U200	XN461(1), XN462(4), XM431(1), XM463(3), XM462(1)	5
3	30U1-U2020-U202	XN431(1), -(2), XN463(2)	3
4	30U1-U2120-U202	XM433(2)	1
5	30U1-U2120-U222	XM463(2)	1
6	30U1-U2120-U222	XM461(1)	1
7	30U1-U2000-U200	XN433(2)	1
8	30U1-U2021-U000	XM434	1
9	30U1-U2025-U202	XM462(1), XN461(2)	2

SERIES III.

In Series III data on eleven hams obtained from different sources, all of which have been aged for approximately twelve months, are presented. Ham No. 3205, Series O, was from a full-fed lot of experimental animals. This was an excellent ham in appearnace, odor, and color. There was very little mold present with none of the white mold that has a tendency to follow the connective tissue into the interior of the ham. (The mate to this one, which was cooked, was a very good ham). During the twelve months aging it had lost 17.5 per cent in weight and now weighed 17.9 pounds.

Hams Nos. 3170 and 3217, Series P and R, respectively, were from a slow-fed lot of animals. Ham P had not aged properly; it was contaminated with a whitish mold which had grown down into the ham; on the side of the bone opposite the biceps femoris muscle, were some grayish green spots which looked as if the ham had started to spoil; the odor was musty. It had lost 21 per cent , and weighed when used 17.7 pounds.

Ham R, also of the same series of slow-fed hogs, had lost only 17.4 per cent during the twelve months aging, and weighed 17.5 pounds when used. This latter was an unusually good ham from every standpoint. There was present a rather small amount of mold; the fat was quite

firm and of a good color. A white flecking was noted in the lean portion. This material was a crystaline precipitate and was more or less irregularily distributed thruout the lean portions of the ham. This seems to be characteristic of properly aged hams. Supplee(1931) has identified this substance as pure tyrosin.

The two hams from the Eastern Shore of Maryland, hams Series IES and 2ES, were very large ones. The lean was of a good red color, well marbled with fat and showed some of the white flecking. A part of the fat and the bone had quite a rancid odor. The lean had a peculiar odor, suggesting something of a damp, musty granary odor plus a certain amount of rancidity.

The two hams designated Series 1WO and 2Wo were from Howard County, Maryland. These hams were characterized by being trimmed with a very long butt. The hogs had been brought to too high a finish and the external layer of fat was out of proportion to the lean present; especially was this true of ham 2Wo. Both showed some white flecking. They had the pleasing odor of a well cured and aged ham. The color of the lean was a good, uniform, dark red and that of the fat, a reddish white.

Ham 1Cr from Prince Georges County, Maryland, had considerable mold on its face. The color of the cut

lean surface was good, with the exception that there was a bright red streak following about an inch below the face surface. It had the appearance and odor of a desirable ham. There were eight samples taken from this ham; samples 5 to 8 were taken from the butt end from positions corresponding to those used for sampling the shank end. They are described elsewhere.

The last three hams in Series III were Joyner's Smithfield hams, usually made from soft pork. Hams 1Sm and 2Sm were typical Smithfield hams, with the deep, dark red color of the lean and the peculiar "clearing" of the fat layer, giving the fat a light amber color. White flecking was present all thru the lean portions. Two of the hams had developed the pleasing odor of properly aged products. This was not true of ham 3Sm. Here, there was no white flecking present. This ham was considerably shrunken; the lean was hard and dry; the fat was very oily and the odor was rather "flat". In fact it was an undesirable product.

Table VI gives the bacterial counts per gram for the hams of this series. It is interesting to note how uniformly low the counts run.

TABLE VI.

Bacterial Counts per gram for hams from different sources which have aged approximately 12 months
(Plate counts in triplicate, shake agar culture counts in duplicate)

Sample No. *	Ham 3205, full fed : Series No. O			Ham 3170, slow fed : Series No. P			Ham 3217, slow fed : Series No. R			Eastern Shore Ham : Series No. 1 E S			Eastern Shore Ham Series No. 2 E S		
	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture
I (1)	25	--	75	1450	525	1125	--	25	--	--	--	--	--	--	--
(2)	25	50	--	1350	575	850	--	--	--	--	--	--	12	6	--
(3)	50	--		900	675		--	--	--	12	--		--	--	
II (1)	500	25	200	--	--	--	--	25	--	12	--	--	--	--	--
(2)	475	--	25	--	25	--	25	--	--	6	--	--	6	--	--
(3)	375	25		--	25		--	--	--	12	6		--	12	
III(1)	--	--	50	75	--	150	--	--	numerous	--	--	--	20	--	--
(2)	75	25	50	50	25	50	25	--	pin mints	6	--	--	6	--	--
(3)	100	25		100	50		--	25		--	--		--	--	
IV (1)	25	50	25	25	125	425	--	--	--	875	597	320	10st	--	lost
(2)	25	25	--	50	175	200	--	--	--	860	630	340		20	6
(3)	25	--		100	275		25	--	--	825	870		25	6	

* I, II, etc. represent different samples taken as described on p.

TABLE VI. - Continued

Bacterial Count per gram for Hams from different sources which have aged approximately 2 months
(Plate counts in triplicate, shake agar culture counts in duplicate)

Sample	Wolfe Ham Series No. 1 Wo.			Wolfe Ham Series No. 2 Wo.			Crandall Ham Series No. 1 Cr.			J.Smithfield ** Series No. 1 Sm.			J.Smithfield ** Series No. 2 Sm.			J.Smithfield ** Series No. 2 SM.		
	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture
I (1)	-	6	-	-	12	12	18	37	125	-	-	-	100	-	25			
(2)	-	-	12	-	6	-	-	44	125	-	-	lost	25	-	-	lost		
(3)	6	-		-	12		12	25		-	-		25	50				
II (1)	81	44	62	-	-	-	6	-	-	-	-	-	25	-	-	30	25	25
(2)	31	56	62	12	18	-	-	-	-	-	250	-	25	25	-	50	25	12
(3)	62	31		-	6		-	6		-	25		25	-	75	25	18	
III (1)	6	-	12	-	18	-	6	-	12	25	-	-	25	-	25	-	-	50
(2)	18	-	12	-	25	-	6	12	-	-	-	-	-	50		12	6	25
(3)	18	6		-	6		6	-		-	25		-	25		18	6	
IV (1)	31	-	25	12	62	-	18	-	-	25	25	-	Pin pts.			-	6	-
(2)	6	12	37	12	45	-	106	6	-	-	-	25	Numerous			-	-	-
(3)	25	-		12	37		-	-		25	-					-	6	-
V (1)	131	137	62	-	25	62	25	-	-							12	-	25
(2)	125	194	162	62	18	25	325	-	-							-	-	-
(3)	181	100		18	12		485	-								25	12	
VI (1)	56	37	-	6	18	-	-	6	-							-	-	-
(2)	50	25	-	-	-	-	-	12	-							-	-	-
(3)	50	44		-	25		6	12								6	6	
VII (1)	-	-	-	6	-	-	-	-	-							-	6	12
(2)	-	-	-	-	-	-	-	-	-							12	6	-
(3)	-	6		6	25		-	6								6	-	
VIII (1)							-	-	-									
(2)							6	-	-									
(3)							-	-										

* I, II, etc. represent different samples taken as described on p.

** Joyner's Smithfield

The organisms isolated are classified according to their Index Numbers in Table VII. Of the 207 cultures studied, 62 or 30 per cent of them were aerobes, while 145 or 70 per cent proved to be facultative anaerobes. The majority of the organisms, or 88.6 per cent of those isolated, were in the Micrococci group; 23.6 per cent were aerobes and 65 per cent were facultative anaerobes. The Micrococci are grouped according to their sugar reaction as follows:

Organism producing acid in	Aerobes		Facultative Anaerobes	
	Number of Cultures	% of Aerobes	Number of Cultures	% of F. Anaerobes
1. Glucose	15	30	25	18
2. Glucose and Sucrose	15	30	52	38
3. Glucose, sucrose and lactose	11	22	46	34

Of the cultures listed in Table VII, Series III, only 7 are rods. These vary in their morphological characteristics; 6 of them are Gram-positive, but otherwise, much variation was noted. There are 9 cultures listed as diplococci. These varied in their sugar reaction from the production of no acid to the production of acid in all three sugars

studied. In this series there was also noted 8 organisms which did not stain definitely Gram-positive. A large yeast was consistently isolated from ham O. These cultures were not included in the counts given.

TABLE VII

CLASSIFICATION BY INDEX NUMBERS OF ORGANISMS FROM HAMS FROM DIFFERENT SOURCES WHICH HAVE AGED APPROXIMATELY 12 MONTHS

Class	Index Number	Culture Number	Total
1	30U1-U1020-U200	2ES421,2ES423,2ES424(2) -(3), 1Wo424(1), -(2), 1Wo425(1), 1Cr422, 1Sm423(1) 1Sm424(2), -(3), 3Sm422(2)	12
2	30U1-U1020-U202	R422, 1ES422(1), 1ES424(2), -(2a), 2ES424(1), 1Wo426(4) 2Wo424(3), 2Wo427, 1Cr423	9
3	30U1-U1020-U220	3Sm424, 1Wo425(4)	2
4	30U1-U1020-U222	1ES424(4), 3Sm427(1)	2
5	30U1-U1120-U000	3Sm426	1
6	30U1-U1120-U202	1ES423, 1ES424(1), -(3) 2Wo425(1), 2Sm422(2), 2Sm423	6
7	30U1-U1120-U222	1Wo423(3), 2Wo426(1), 1Cr421(1), -(2), 2Sm422(3)	5
8	30U1-U1U20-U200	1Wo422(3), 1Sm423(3)	2
9	30U1-U1000-U200	3Sm422(1)	1
10	30U1-U1000-U000	P423(3), 1Wo, 425(2)	2
11	30U1-U1000-U222	0424(1), 0151,P424(1),R422	4
12	30U1-U1020-U000	1Wo422(1), -(2), 3Sm423(2),	3
13	30U1-U2U20-U200	X1ES464(5), X1Wo432(1), X1Wo462(4), X1Cr431(1), X1Cr465(1), X1Cr466(1),-(2), X2Sm431, X3Sm464(2),	9
14	30U1-U2020-U200	X0434(1), X0464(3), XP461(1), -(2), -(3), XP462(1), -(2), -(3), XP463(1), XP464(1) X1Wo432(2). X3Sm433(1) -(2), X3Sm462(1)	14

TABLE VII (Continued)

Class	Index Number	Culture Number	Total
15	30U1-U2U20-U202	X1ES463(1),X1ES464(2),-(3), -(4), X1Wo434,X1Wo435(1) -(2), X1Wo461, X1Wo463, X1Wo464(1), X2Wo467(2). X2Wo465(1), X2Wo431, X1Cr431(2),-(3),-(4),X1Cr461(2), -(4), -(5), X1Cr464, X1Cr466(3) X1Cr467, X3Sm463(2), X3Sm467(1), -(2), -(3),	27
16	30U1-U2020-U202	XP433(1), -(2), -(3), XP434(1), XP461(5), XR461(1), X2Wo461 (3), X1ES434(3), X2ES464(2), X2Wo461(1), X2Wo462(2). X2Wo463(1), X2Wo464(1) -(2), X3Sm461(1),	15
17	30U1-U2120-U202	X1ES434(1), X2ES463(3), X1Sm462(1)	3
18	30U1-U2U20-U220	X3Sm464(1)	1
19	30U1-U2020-U220	$XO_a151(1)$, X2Sm461(1)	2
20	30U1-U2120-U220	X0464(5), X1ES434(2),-(4),	3
21	30U1-U2U20-U222	X1ES462(3), X1Wo462(1), X1Wo465(2), -(3), X1Wo466(1), -(2), X2Wo462(1), X2Wo464(3), X2Wo466(3), X1Cr461(1) X1Cr462, X1Cr465(2). X3Sm464(3), X3Sm465	14
22	30U1-U2020-U222	XP431(3), X0464(3a) X2ES462(2), -(4), -(5), X2Wo461(2), -(4), -(5), X1Sm461, X1Sm462(2), -(3), X2Sm433(2), X2Sm462(1) X3Sm462(2), -(4)	15
23	30U1-U2120-U222	XP434(3), XR464, XP464(5), X2ES462(1), -(6), X2ES463(2) -(4), X2ES464(1), -(3), X1Sm462(4), X1Sm464(2), X2Sm462(2)	12
24	30U1-U2100-U202	X0464(2), XP464(3)	2
25	30U1-U2100-U222	X0431(3)	1
26	30U1-U2120-U002	XR463(1)	1

TABLE VII (Continued)

Class	Index Number	Culture Number	Total
27	30U1-U2000-U200	XO464(6), X1Sm434	2
28	30U1-U2000-U202	XO463(1), XR433(1),-(2) X1Sm464(5), X2Sm463(1)	5
29	30U1-U2U00-U220	X1Wo466(4),X2Wo466(1), X1Wo467, X2Wo435(1)	4
30	30U1-U2U00-U222	X1ES461,X2Wo435(3)	2
31	30U1(?)U2020-U222	XP464(4),X1Wo465(1),X2Wo435(2) X1Cr461(3), X1Cr463,X3Sm462(3) X3Sm463(1),X3Sm466	8
32	50U1-U1120-U222	2Wo425(2)	1
33	50U1-U1120-U200	1Cr424(3)	1
34	50U1-U1120-U202	1Cr428, 2Sm421(1)	2
35	50U1-U2020-U200	XP464(2)	1
36	50U1-U2120-U222	X2Wo463(2)	1
37	50U1-U1020-U200	1 Sm424(1)	1
38	30U1-U2005-U222	1Sm464(1)	1
39	30U1-U2128-U222	XO464(4)	1
40	20U1-U2000-U222	XO_a151(2)	1
41	20U1-U1005-U000	3Sm427(2)	1
42	20U1-U1020-U220	1Wo424(3), 1Wo425(3),-(5)	3
43	20U1-U1020-U202	0421(4), 0423(1),R423(1),-(2)	4

Yeast isolated from Ham O.

SERIES IV.

In this series three of the hams were from experimentally fed hogs and had been allowed to age for 24 months. The other ham was a farm-cured product which had aged for 36 months. Ham No. 3157 was from a full-fed series of animals and is designated in the tables and discussion as S. This was one of the most desirable hams used for analysis. It had that elusive, pungent, cheesy aroma which is so sought after in these aged hams. At the same time the texture of the lean was not too dry and there was present some of the white flecking. There was only a small amount of mold present. The color of the freshly cut lean surface was a fairly uniform light red, with a slight darkening towards the face; the fat was reddish white. The ham had lost 20.5 per cent during the twelve months aging and weighed 16.3 pounds when used.

Hams Nos. 3158 and 3159, Series designations T and U respectively, were from a slow-fed lot of experimental animals, which were slaughtered at the same time as the animals from the above full-fed lot, hence, they were much lighter in weight. Ham T weighed when used 8.5 pounds and ham U only 7.75 pounds. The former had lost 28 per cent, while the latter, 29 per cent during the

24 months aging. There was considerable mold on the face of each of these hams. Neither of them were especially desirable products. Because of the lack of finish given the hogs before slaughter, the small hams apparently dryed out more than the heavier ones, thus resulting in hard and dry lean portions. The aroma was somewhat stale and musty.

Ham 2Cr from Prince Georges County, Maryland, had aged for approximately 36 months. It was considerably shrunken in appearance; and its face was rather thickly covered with cayenne pepper. The cut surface showed the lean to be a fairly uniform dark red; the fat was reddish white with a yellowish tinge. There was some white flecking present in the lean and the ham had an old, rancid odor with a suggestion of creosote, which was somewhat different from that of smoke.

Table VIII gives the bacterial counts, aerobic and anaerobic, per gram for the hams of Series IV. It is of interest to note the large number of plates on which no growth was secured.

TABLE VIII.

Bacterial Count per gram for 3 hams which have aged 24 months, and one 36 months.
(Plate counts in triplicate, shake agar culture counts in duplicate)

Sample No.	Ham 3157, full fed 24 mo. ham. Series No.S.			Ham 3158, slow fed 24 mo. ham. Series No. T.			Ham 3159, slow fed 24 mo. ham. Series No. U.			Crandall ham, 36 mo. old. Series No. 2 Cr.		
	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture	Aerobic Plate	Anaerobic Plate	Shake Agar Culture
I (1)	-	-	-	194	131	212	50	65	50	12	12	-
(2)	6	-	-	281	194	212	31	122	50	25	18	12
(3)	-	-		231	137		12	62		25	25	
II (1)	-	6	-	-	6	-	44	18	100	6	-	-
(2)	-	-	-	-	-	-	25	18	-	6	-	-
(3)	6	-		-	25		12	lost		12	-	
III (1)	-	6	12	18	-	-	-	18	-	6	-	-
(2)	-	-	-	-	-	-	-	-	-	6	-	-
(3)	-	-		-	6		-	6		12	6	
IV (1)	100	94	86	-	-	-	-	-	-	75	-	-
(2)	100	88	156	-	6	-	31	-	-	6	-	-
(3)	112	88		18	6		12	-		6	-	
V (1)							-	-	-	-	6	-
(2)							-	-	-	-	-	-
(3)							12			-	-	
VI (1)							-	-	-	-	-	-
(2)							-	-	-	-	-	-
(3)							-	-		-	-	
VII (1)							-	-	12	6	6	12
(2)							-	-		-	6	12
(3)							12	6		12	12	

- In Table IX the cultures isolated are classified by their Index Numbers. Again the Micrococci group predominated. Of the 59 cultures studied 88 per cent were micrococci; 23 or 39 per cent, aerobes and 29 or 49 per cent were facultative anaerobes. These micrococci are grouped according to their sugar reactions as follows:

Organisms producing acid in	Aerobes		Facultative Anaerobes	
	Number of Aerobes	% of Aerobes	Number of Cultures	% of F.Anaerobes
1. Glucose	5	23	3	10
2. Glucose and sucrose	5	23	4	14
3. Glucose, sucrose and lactose	13	57	20	70

Beside the micrococci listed there were two micrococci that gave doubtful Gram reactions, and 4 large beaded rods and one diplococcus were noted.

TABLE IX

CLASSIFICATION BY INDEX NUMBERS OF ORGANISMS FROM THREE HAMS AGED 24 MONTHS AND ONE HAM AGED 36 MONTHS

Class	Index Number	Culture Number	Total
1	30U1-U1020-U200	U421(1), 2Cr422(1)	2
2	30U1-U1120-U200	U427	1
3	30U1-U1I20-U200	U421(3)	1
4	30U1-U1000-U200	U422(2)	1
5	30U1-U1020-U202	T423(1),-(3)	2
6	30U1-U1U20-U202	U422(6)	1
7	30U1-U1000-U202	S422	1
8	30U1-U1020-U222	S424(3),S424(1),-(2), T423(4),U421(4),U422(4)	6
9	30U1-U1120-U222	2Cr426(3)	1
10	30U1-U1U20-U222	U421(1),-(2),U424(1),-(2)	4
11	30U1-U1000-U222	U422(5)	1
12	30U1-U2U20-U200	XU431(1),XU461(1),-(3)	3
13	30U1-U2U20-U202	XT434,XT463,XU431(2)	3
14	30U1-U2U20-U222	XS462, XS463, XT462(1), X2Cr467	4
15	30U1-U2020-U222	XS434(3), XT464	2
16	30U1-U2120-U222	XS434(1), XS464(2), -(3),-(4), XT462(3), XU462(2), XU463, XU464,	8
17	30U1-U2100-U202	XS434(2)	1
18	30U1-U2100-U220	XU462(3)	1
19	30U1-U2100-U222	XU462(1), -(4)	2
20	30U1-U2000-U222	XT462(2),XU467,XU437, X2Cr465	4

TABLE IX - (Continued)

Class	Index Number	Culture Number	Total
21	30U1-U2U00-U000	XT461(2)	1
22	30U1(?)-U2020-U222	XS461(1), XU461(2)	2
23	50U0-U1120-U202	S421(1),-(a), 2Cr423(1),-(2)	4
24	20U1-U1020-U220	T424	1
25	30U1-U1026-U222	U425	1
26	30U1-U1128-U202	T423(2)	1

A summary of the classification by Index Numbers of all cultures isolated in Series I to IV inclusive, is presented in Table X. The discussion of this data in connection with the quantitative counts from the individual samples of ham will be found under the heading "Discussion".

TABLE X.

A summary of the classification by Index numbers of all cultures isolated in Series I. to IV., inclusive

Source	TOTAL cultures isolated	Gram-Positive Micrococci						Rods				Gram-Negative (?) Micrococci				Diplococci			
		Aerobes		Facultative Anaerobes		Total Micrococci		Aerobes		Facultative Anaerobes		Aerobes		Facultative Anaerobes		Aerobes		Facultative Anaerobes	
		Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Table III.	130	48	37	62	49	110	86	1	0.7	10	7.6	1	0.7	8	6.1	0	0	0	0
Table V.	16	2	12	14	88	16	100												
Table VII.	207	49	23.6	134	65	183	88.6	5	2.4	2	0.9	0	0	8	3.8	8	3.8	1	0.48
Table IX.	59	23	39	29	49	52	88	4	8.5	0	0	0	0	2	3.4	1	1.7	0	0

SERIES V.

In this series of experiments the object was to determine the lipolytic activity of each of the samples which were taken from the five hams just out of cure. The method used in making these tests is described on page 23. Tests and controls on each sample were run in triplicate. Controls differed from the tests only in the fact that for the former the meat extract was boiled to kill the enzymatic activity of the sample before adding it to the oil. Both sets were incubated at 37°C for 96 hours. Table XI gives the results of these tests, listing the number of cc. of N/10 NaOH which was required to neutralize the free-fatty acids formed by 2 cc. of the 1 to 12.5 water-dilution of the macerated ham sample added to 10 cc. of highly refined cotton-seed oil. This value is converted to the per cent of oleic acid which would have been produced if a one gram sample had been used instead of the 2 cc. of the 1 to 12.5 dilution. The values for hams V and W which were "standard cured" hams, run consistently lower and with less variation than do the values for the Swift Premium hams. Little consistency is noted in the values obtained for samples from corresponding positions in the various hams.

TABLE XI.

Giving the number of cc. N/10 NaOH required to neutralize the free fatty acids formed by 2 cc of 1-12½ dilution of sample from hams just "out of smoke", also % oleic acid on basis 1 gram of ham

Ham No.	Sample No.	No. cc N/10 NaOH required to neutralize free fatty acids formed from 1-6¼ dil.	% Oleic Acid Basis 1 gram
Beltsville Ham	V I	.10	.2200
	V II	.12	.2640
	V III	.40	.8800
	V IV	.07	.1540
	V V	.27	.5940
	V VI	.13	.2860
	V VII	.35	.7700
Beltsville Ham	W I	.13	.2860
	W II	.23	.5060
	W III	.17	.3740
	W IV	.13	.2860
	W V	.20	.4400
	W VI	.20	.4400
	W VII	.27	.5940
Swift Premium	1 SP I	.30	.6600
	1 SP II	.17	.3737
	1 SP III	-.14	-.3081
	1 SP IV	.06	.1320
	2 SP I	.40	.8800
	2 SP II	.56	1.2319
	2 SP III	.87	1.9137
	2 SP IV	.27	.5937
	3 SP I	.44	.9680
	3 SP II	.27	.5940
	3 SP III	.02	.0440
	3 SP IV	.03	.0660

SERIES VI.

This series differed from Series V only in the fact that the age of the hams are different. Ham M was from a full-fed hog and had been aged for 4 months, while ham N was from a slow-fed animal and had aged for 8 months. The values reported in Table XII are of interest. Some of them are higher than those obtained from any other samples used irrespective of the source or age of the ham. In view of the fact that these were the only hams of these specific ages analyzed no conclusions can be drawn from the results stated.

TABLE XII.

Giving the number of cc. of N/10 NaOH required to neutralize the free fatty acids formed by 2 cc. of 1 to 12½ dilution of sample from hams aged 4 and 8 months respectively

Ham No.	Sample No.	No. cc. N/10 NaOH required to neutralize full fatty acids formed by 1-6¼ dil.	%oleic acid Basis 1 gram
3229	M I	.40	.8581
4 mo.	M II	.20	.4400
full-fed	M III	1.35	2.9700
right	M IV	.90	1.9800
ham			
3213	N I	.50	1.1000
8 mo.	N II	2.00	4.4000
slow-fed	N III	.40	.8800
right ham	N IV	2.10	4.6190

SERIES VII.

In Series VII the object was to determine the amount of lipolytic activity of the samples from hams which had aged for 12 months. There were 11 hams tested in this series. Descriptions of these hams are to be found under Series III. The results of the values found for the lipase activity of the individual samples from various hams of this series are given in Table XIII. None of the values obtained in this series are as high as some of those found in Series VI, but they are consistently higher than those obtained in Series V, with the exception of the values for ham 1ES.

TABLE XIII

Giving the number of cc. N/10 NaOH required to neutralize the free fatty acids formed by 2 cc. of 1 to 12½ dilution of sample from hams from different sources, all having been aged approximately 12 months; also % oleic acid on basis 1 gram of ham.

Ham No.	Sample No.	No. cc. N/10 NaOH required to neutralize free fatty acids formed from 1-6¼ dilution	% Oleic Acid Basis 1 gr.
3217 Slow fed rt.ham	R I	.55	1.2100
	R II	.70	1.5400
	R III	.77	1.6940
	R IV	.43	.9460
3170 Slow fed rt. ham	P I	1.90	4.1700
	P II	.26	.5719
	P III	.80	1.7600
	P IV	.47	1.0337
3205 Full fed rt. ham	O I	2.07	4.5537
	O II	.47	1.0337
	O III	1.26	2.7719
	O IV	.40	.8800
Eastern Shore Ham 12 mo.	1 ES I	.13	.2860
	1 ES II	.04	.0880
	1 ES III	.10	.2200
	1 ES IV	.16	.3520
Eastern Shore Ham	2 ES I	.53	1.1660
	2 ES II	.75	1.6500
	2 ES III	.00	0.0000
	2 ES IV	.42	.9240
Wolfe Ham	1 Wo.I	.86	1.8920
	1 Wo.II	1.00	2.2000
	1 Wo.III	.77	1.6940
	1 Wo.IV	.53	1.1660
	1 Wo.V	.47	1.0340
	1 Wo.VI	.54	1.1880
	1 Wo.VII	.80	1.7600
Wolfe Ham	2 Wo.I	.11	.2420
	2 Wo.II	.13	.2860
	2 Wo.III	.20	.4400
	2 Wo.IV	.30	.6600
	2 Wo.V	1.03	2.2660
	2 Wo.VI	.23	.5060
	2 Wo.VII	.30	.6600
Crandall Ham	1 Cr. I	.16	.3520
	1 Cr.II	.14	.3080
	1 CR.III	.43	.9460
	1 Cr.IV	.23	.5060
	1 Cr.V	.03	.0660
	1 Cr.VI	.10	.2200
	1 Cr.VII	.36	.7920
Joyner's Smith-field	1 Sm.I	0.00	0.0000
	1 SM.II	.34	.7481
	1 SM.III	.23	.5065
	1 SM.IV	.54	1.1881
Joyner's Smith-field	2 Sm.I	.47	1.0337
	2 Sm.II	.54	1.1881
	2 Sm.III	.13	.2862
	2 Sm.IV	.06	.1320
Joyner's Smith-field	3 Sm.I	.72	1.5840
	3 Sm.II	1.00	2.2000
	3 Sm.III	.21	.4620
	3 Sm.IV	.22	.4840
	3 Sm.V	.64	1.4080
	3 Sm.VI	.38	.8360
	3 Sm.VII	.27	.5940

SERIES VIII.

The object of this series was to determine if the samples from hams which had aged for 24 months or longer, still possessed active lipolytic properties. The results of these tests are found in Table XIV. It will be noted that while the values obtained for this series are low, they are slightly higher than the majority of values obtained in Series V, but they are considerably lower than the majority of values for the samples in Series VI.

TABLE XIV.

Giving the number of cc. N/10 NaOH required to neutralize the free fatty acids formed by 2 cc of 1-12½ dilution of sample for hams, 3 of which have aged 24 months and 1 36 months

Ham No.	Sample No.	No. cc N/10 NaOH required to neutralize free fatty acids formed by 1-6¼ dil.	% Oleic acid basis 1 gram
3157	S I	.10	.2200
full-fed	S II	.37	.8140
right	S III	.10	.2200
ham	S IV	.48	1.0560
3158	T I	.25	.5500
Slow-fed	T II	.37	.8140
right	T III	.38	.8360
ham	T IV	.14	.3080
3159	U I	.27	.5940
Slow-fed	U II	.23	.5060
left ham	U III	.40	.8800
	U IV	.20	.4400
	U V	.20	.4400
	U VI	.40	.8800
	U VII	.22	.4840
Crandall	2 Cr. I	.13	.2860
Ham	2 Cr. II	.20	.4400
3 yrs.	2 Cr. III	.37	.8140
old	2 Cr. IV	.70	1.5400
	2 Cr. V	.25	.5500
	2 Cr. VI	.27	.5940
	2 Cr. VII	.30	.6600

The differences between the amounts of N/10 NaOH required to neutralize the free-fatty acid formed in the test and control flasks of individual samples during the lipase experiment (as stated in Tables XI to XIV, inclusive), do not give the whole picture of the situation. Tables XV and XVI are presented as examples of the values obtained for the individual tests run on samples from two different hams. (The other detailed tables in this series of experiments may be found in a Progress Report of this work to the Maryland Experiment Station (1931)). These two tables were chosen as representatives of the two methods used in preparing the meat extract from the control tests. In Table XVI the extract was boiled in a 1 X 8 inch test tube over a Bunsen burner for one minute. There will be noted in this case a decided increase in the values for the controls (Labeled (1B), (2B), etc.) in samples SIII and SIV over those for samples SI and SII. The only explanation which could be found for this was that evidently all of the lipolytic activity of the preparation had not been destroyed by this amount of heat. Therefore, steaming the extract for 1 hour in live steam was tried. When this was done such wide variations was not found in the

values obtained for the individual control tests, as will be noted in Table XV, The differences of the averages of the triplicate checks on the tests and controls obtained for each sample for the two hams are quite similar. Controls for the samples from hams, T, U and 2Cr in Table XIV were steamed rather than boiled. It will be noted here that the differences obtained for the individual samples from these hams are more nearly uniform than those for ham S.

TABLE XV.

Result of Lipase test run on Beltsville Ham, cured by Standard Method. Series No. V.

Sample No.	No.co. of N/10 NaOH for 1-6¼ dilution	A verage CC.	Difference CC.	% Oleic Acid Basis 1-6¼ dilution	% Oleic Acid Basis 1 gram
V I (1)	1.00				
(2)	.95	.93			
(3)	.85		.10	.03520	.22000
(1B)	.80				
(2B)	.80	.83			
(3B)	.90				
V II (1)	.70				
(2)	.80	.80			
(3)	.90		.12	.04224	.26400
(1B)	.75				
(2B)	.60	.68			
(3B)	.70				
V III (1)	1.00				
(2)	1.00	1.17			
(3)	1.50		.40	.14080	.88000
(1B)	.80				
(2B)	.70	.77			
(3B)	.80				
V IV (1)	.70				
(2)	.80	.73			
(3)	.70		.07	.02464	.15400
(1B)	.60				
(2B)	.65	.66			
(3B)	.60				
V V (1)	1.00				
(2)	.90	.92			
(3)	.85		.27	.09504	.59400
(1B)	.55				
(2B)	.70	.65			
(3B)	.70				
V VI (1)	.70				
(2)	.80	.73			
(3)	.70		.13	.04576	.28600
(1B)	.60				
(2B)	.60	.60			
(3B)	.60				
V VII (1)	1.00				
(2)	.80	.88			
(3)	.85		.35	.12320	.77000
(1B)	.50				
(2B)	.50	.53			
(3B)	.60				

TABLE XVI.

Result of lipase test run on Ham 3157, Series No. s, full fed, aged 24 months.

Sample No.	No.cc. of N/10 NaOH for 1-6¼ dil.	Average	Difference	% Oleic Acid Basis 1-6¼ dil.	% Oleic Acid Basis 1 gram
S I (1)	1.30				
(2)	1.20	1.30			
(3)	1.40		.10	.03520	.22000
(1B)	1.20				
(2B)	1.30	1.20			
(3B)	1.10				
S II(1)	1.80				
(2)	1.60	1.68			
(3)	1.65		.37	.13024	,81400
(1B)	1.35				
(2B)	1.35	1.31			
(3B)	1.25				
S III(1)	1.95				
(2)	2.10	2.00			
(3)	2.00		.10	.03520	.22000
(1B)	2.05				
(2B)	1.70	1.90			
(3B)	1.95				
S IV (1)	2.30				
(2)	3.00	2.53			
(3)	2.40		.48	.16896	1.05600
(1B)	1.95				
(2B)	2.10	2.05			
(3B)	2.10				

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IV

DISCUSSION.

In a consideration of the quantitative bacterial counts from aerobic and anaerobic plates and from shake agar culture tubes, the number of plates and tubes yielding growth shows a decrease as the age of the hams increase. The number of colonies found per plate or tube also tends to decrease with the aging of the ham. A summary of the number of aerobic and anaerobic plates and of shake agar culture tubes which yield growth is found in Table XVII.

TABLE XVII.

Number of Aerobic and Anaerobic plates and Shake Agar culture tubes yielding growth.

	Aerobic Plates			Anaerobic Plates			Shake Agar Cultures tubes		
	Number	Yielding Growth		Number	Yielding Growth		Number	Yielding Growth	
		Number	%	Plates	No.	%	Tubes	No.	%
Table II	78	58	75	78	50	64	52	37	71
Table IV	12	9	75	12	8	66	12	8	66
Table VI	171	102	60	171	93	55	114	46	30
Table VIII	66	34	52	66	30	46	44	12	28

TABLE XVIII

Number and percent of aerobic and anaerobic plates and of Shake Agar cultures tubes yeilding 1 to 4 colonies.

	Aerobic Plates		Anaerobic Plates		Shake Agar Culture tubes	
	Number Yielding 1 to 4 colonies	% of total aerobic plates yielding growth	No. Yielding 1 to 4 Colonies	% of total anaerobic plates yielding growth	No. Yielding 1 to 4 colonies	% of total S.A.C. tubes yielding growth
Table II	29	29	26	52	22	60
Table IV	6	55	6	75	2	16
Table VI	41	40	69	74	22	48
Table VIII	23	68	18	60	7	58

The data taken from Table IV and included herein, may be somewhat misleading, because there were only two hams in this series, one of which had aged for 4 months and one, for 8 months. Aerobic growth was secured on 75 per cent of the plates from hams just out of cure, on 60 per cent of the plates from hams aged 12 months, and on 52 per cent of the plates from hams which had aged for 24 months or longer. The decrease of growth found in the case of the anaerobic plates was not so great; growth resulted in 64 per cent of the plates representing hams just out of cure and 46 per cent of the plates from the oldest hams. The shake agar culture tubes showed the greatest decrease of all. The freshly cured hams yielded 71

per cent growth; the 12 months series, 40 per cent growth, while the group of hams ages 24 months or longer yielded only 28 per cent growth.

This is the trend which would naturally be expected, because as the hams age their moisture content decreases. There are at least two factors which contribute to this decrease; one is the moisture loss due to evaporation during the aging process, and the other is due to certain chemical changes which are continually taking place in the hams as they age. Supplee (1931) has shown by chemical analysis that the percent of free-fatty acids in the hams increases as the hams age; this hydrolysis of fats to free-fatty acid and glycerol in the meat-fat does not reach an equilibrium during 24 months of aging. In the complete hydrolysis of a molecule of fat, three molecules of water are required. This may account for only a small amount of the moisture loss, but at least a part of the free-water is converted into chemically bound-water in this process of hydrolysis. As the moisture content of the hams decreases, the salt concentration naturally increases, and no doubt, there is a change in the hydrogen-ion concentration, all tending to make conditions less favorable for the growth of micro-organisms.

The data in Table XVIII (p.68), gives a summary of the number and percent of aerobic and anaerobic plates and shake agar culture tubes yielding from 1 to 4 colonies. It will be noted that for the aerobic and anaerobic plates this number increased as the hams aged. The number of aerobic plates increased from 29 per cent for hams just out of cure to 68 per cent for hams which had aged for 24 months or longer. In the case of the anaerobic plates the increase was not so great. For the hams just out of cure 52 per cent of the anaerobic plates showed only 1 to 4 colonies per plate. The hams which had aged for 12 months showed 74 per cent of the plates in this group, while for the oldest hams used the figure had dropped to 60 per cent. In the case of the shake agar culture tubes the number of tubes falling within this group was much more nearly constant, as will be noted from Table XVIII, varying from 48 per cent for the 12 months hams to 60 per cent for the hams just out of cure. The figures in this Table are not consistent in their trend as are those shown in Table XVII, but they do show that 48 per cent of all the aerobic plates showing growth yielded from 1 to 4 colonies per plate; that 65 per cent of the anaerobic plates and 43 per cent of all the shake agar culture tubes yielding growth yielded only 1 to 4 colonies per plate.

A summary of the classification by Index Numbers of all the cultures isolated from all the hams is reported in Table X. The factors which are of particular interest from this table are:

(1) The highest percentage of the total cultures isolated was found to fall in the Micrococci group. A medium small staphlococcus seemed to be the predominating type. On pages 29, 35, 42 and 50 are given glucose, sucrose and lactose sugar reactions of the Gram-positive micrococci isolated. None of them produced gas in any media. Table X shows that 86 per cent of the cultures isolated from hams just out of cure were in this group, with 37 per cent as aerobes and 49 per cent as facultative anaerobes. From the hams which had aged for 12 months, 88.6 per cent of the cultures isolated were Gram-positive micrococci, while for the 24 months old hams 88 per cent were in this group. Of the former 23.6 per cent were aerobes and 65 per cent, facultative anaerobes. Of the latter 39 per cent were aerobes and 49 per cent, facultative anaerobes. From the 4 and 8 months hams, only Gram-positive micrococci were isolated, with the exception of a few cultures of a large yeast which was isolated from ham N.

(2) Very few rods were isolated during the course of the study, and of those isolated, none were spore-formers. The majority of bacilli from hams just out of cure were facultative anaerobes. They were fairly large, beaded bacilli. Those from hams which had aged for 12 months were mostly aerobes and varied more morphologically. They included a fairly large bacillus in chains, a short, fat, round-ended one, a few medium sized bacilli, and one small Gram-negative rod.

(3) A few micrococci which gave a definite Gram-negative and a few which gave an indefinite Gram-reaction, were isolated and studied. Most of these cultures were facultative anaerobes. Time did not permit determining whether or not, these organisms were the same as the sperical, Gram-negative organism which Sturges(1923) isolated from meat curing solutions. Besides the cultures already enumerated there were 10 cultures listed as diplococci, most of which were isolated from the aerobic plates.

These findings seem to be quite at variance with the findings of Reith(1926) and Boyer(1923, 1926), wherein they report a large per cent of anaerobic organisms isolated from hams just out of cure.

Of the hams from animals experimentally raised, given the "standard cure", and aged at the University of Maryland Experiment Station, several were contaminated with a whitish mold which invariably had the tendency to follow the connective tissue down into the interior of the ham. When this occurred the hams had a musty, moldy odor which made them undesirable. This was noted also in the hams which were cooked for palatability tests. This mold was isolated, but the lack of time prevented its identification. There was another mold which appeared on the face of the hams with much greater frequency than the mold just mentioned. This was a dark, greenish gray mold which apparently did not have any particular effect upon the flavor or odor of the ham as it did not seem to penetrate below the surface. This mold produced a large amount of water soluble, reddish purple pigment when grown on Dox agar. When this particular mold was present on the face of the hams in appreciable quantities, there was always a decided darkening of the upper muscle, sometimes to the depth of an inch or more. This seems rather suggestive of the fact, that the soluble pigment formed by this mold may be a factor in causing this darkening. Of course the proof of this awaits the identification of

the mold and then the subsequent inoculation of it on sound, uncontaminated hams. Usually there were also other species of molds present on these hams.

The Maryland farm-cured hams which were analyzed, were free from mold contamination with the exception of the ham 1Cr. There was considerable mold on this ham, but the predominating species seemed to be a blue green mold. Most of these products had been treated with borax after curing to prevent molding of the product. None of the hams from Swift and Company were contaminated with mold.

The lipolytic activity of the water extracts made from the samples taken from hams of different ages and from different sources (as suggested by the results shown in Table XI to XIV, inclusive) is somewhat as follows: (1) the lowest values were obtained in the case of the hams just out of the cure (Table XI): (2) the ham 8 months old gave the highest values of any ham tested, with the 4 months ham a close second to it (Table XII). As there was available only one ham of each of these ages, it cannot be said that this would be true in a majority of the cases. (3) The results (Table XIII) from the eleven hams, all of which had aged for approximately 12 months, show that while all of the values here are lower than some of those found in Table XII, they are as a rule considerably higher than those for the hams just out of cure (Table XI). For ham 1ES and for a few samples from other hams of this group, results fall below the values in Table XI. (4) The hams which have aged for 24 months or longer, show a further decrease in the lipolytic activity as noted by the water extracts made from the samples taken from them, but the results obtained are consistently higher than those from the hams just out of cure.

In Tables XV and XVI it will be noted that there is considerable difference in the number of cc. required

to neutralize the individual tests and control flasks. This is accounted for by the fact that it was necessary to procure the oil in two different shipments. While there was a difference in the total amount of free-fatty acids formed from the oil secured at different times, the values were quite constant for each individual lot. That the difference between the averages of the triplicate tests and control flasks for the same sample of water extract of meat by the different oils did not vary materially, was shown by checks which were run before the new shipment of oil was used. The first tests were made with ordinary commercial Wessen oil. With this the amount of N/10 NaOH required to neutralize the free-fatty acids formed in the individual tests and control flasks, was higher than for the more highly refined cotton-seed oil. This seemed to indicate that there was still present in the Wesson oil some residual natural lipase which was brought into action by the addition of the water of the sample.

From results of experiments in this investigation, the conclusion was reached that at least for the water extracts from samples of cured hams, it was necessary to boil the extract longer than one minute as

Kanitz(1905) suggests, in order to insure the inactivation of the enzyme. . In these experiments the object was to carry the tests to approximate equilibrium in order to try and determine if there was any appreciable lipolytic activity possessed by the tissue of the cured ham. The investigations of Supplee(1931) strongly suggested that there was, and these lipase tests indicate that while there is not such a great amount present, there is some. It would be of interest to employ other tests (as the stalagmometric method used by Dell'Acqua(1930) to estimate lipase content of tissues, or the method used by Willstätter, Waldschmidt-Leitz and Memmen(1924) to express the relation between lipase quantity and degree of saponification) to measure more definitely than has been done here the quantity of lipase present in the tissues of hams of different ages. The working out of a method for measuring the amount of lipolytic activity of the cured ham tissue during the aging process, will possibly give some valuable information which may be used in determining the amount of lipase preparation to be injected in the hams in order to speed up the fat hydrolysis; thus, shortening this step in the aging process. It is not as yet known whether this increase in free fatty acids in aged hams is one of

the factors responsible for the production of the desired flavor and texture in these hams or not, but it is a known fact that the increase does take place during aging.

The question of whether this activity is inherent in the ham tissue itself or whether the micro-organisms present are responsible for a part of the activity, is a field of interesting speculation. Especially is this true, when one considers the fact that lipase activity has already been proven to be a function of a number of our more or less common bacteria (See p. 9). Corran (1929) found that small concentrations of NaCl in the water extract augmented lipolytic activity; this may prove to be quite pertinent to this problem.

It would be of interest and also possibly of practical value to test the micro-organisms isolated from the cured hams of different ages, for their lipolytic activity. Altho the data resulting from this investigation does not seem to indicate any correlation between the number of micro-organisms present, as evidenced by the bacterial count, and the amount of lipolytic activity determined for the sample, there may be such a correlation.

V

SUMMARY.

I. Bacterial counts made by aerobic and anaerobic plates and shake agar culture tubes, on hams just out of cure and on those which had aged for different lengths of time after curing, indicated that as the age of the hams increased the number of micro-organism decreased. As the age of the ham increased, there was an increase in the number of plates and tubes from which growth was not obtained. In general there was also a decrease in the number of colonies present on those plates and tubes upon which growth was obtained.

2. Of the 412 bacterial cultures isolated, 309 or 75 per cent were facultative anaerobes and the remainder were aerobes. These cultures were grouped according to their Index Numbers.

3. Eighty-eight per cent or 361 of the total were Gram-positive micrococci, of which 279 or 80 per cent were facultative anaerobes. Nineteen or 4.6 per cent of the total organisms gave either a Gram-negative or a doubtful reaction. All but one of these were in the facultative anaerobic group.

4. Twenty-two or 5.3 per cent of the total bacterial cultures isolated were rods, none of which were spore formers.

5. Ten cultures of the total were classified as diplococci. Yeasts were isolated from three different hams, one in each Series except Series I, They are not included in the counts given.

6. Molds were isolated from a number of the hams. There were two types which seemed to be of importance in relation to the aging of hams. One of these was a light colored mold which always had a tendency to grow into the interior of the ham giving a musty, moldy odor. The other mold was a grayish green mold that did not penetrate the ham, but produced a reddish purple pigment which filtered into the upper muscle to some extent.

7. Tests for lipolytic activity as determined by the Kanitz method, indicated that in the majority of the hams studied there was some hydrolysis of the fat. The least lipolytic activity took place in hams just out of cure. The results obtained with the hams which had aged 4, 8, and 12 months respectively, were similar. A few of the individual samples from hams 4 and 8 months of age yielded the greatest hydrolysis of fat. The values obtained for the lipolytic activity of the hams which had aged for 24 months or longer were only slightly higher than those obtained from hams just out of cure.

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