A BACTERIOLOGICAL SURVEY OF CERTAIN PROCESSED MEATS AT PACKER AND RETAIL LEVELS

by

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This survey extending over a period of the last two years was initiated at the request of the Bureau of Food Control, Manitoba Department of Health and Welfare. Financial support for the study was obtained from the Federal Department of Health.

In all, a total of over 300 samples were examined. In the first year of the investigation, samples were donated by the various packers and retailers concerned in the study. In the second year, however, all samples examined were provided through the courtesy of the two packing houses involved. This was necessary to ensure the availability of particular code-date packages at the packer and retail level and to provide continuity of sampling during the investigation.

Accordingly, we extend sincere thanks for the sympathetic interest and co-operation to those company personnel involved and to all who so willingly participated in the investigation. We gratefully acknowledge the co-operation of Canada Packers, Swift Canadian, Canada

Safeway, Loblaws, Dominion Stores, The A and P Company and Shop Easy Stores.

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ABSTRACT

Replicate sampling of wieners and cooked ham from the wrapping line of government inspected packing houses revealed considerable variation in bacterial colony counts.

Total counts were higher and variations in count were more marked in wiener samples from Packer X than in those from Packer Y and correlated closely with the presence of large numbers of "pin-point" colonies, predominantly Enterococci. In contrast, only a few of the pin-point colonies on ham from either Packer were members of the Enterococcus group. Correlation was also noted between oxygen permeability of various film wraps; numbers being higher on wieners wrapped in Cryovac from Packer X than on Cello Wrap wieners from Packer X or Packer Y.

Growth change studies indicated frequently a decrease in population numbers with time on plates incubated at 37° C in contrast to an increasing population on plates incubated at 10° C.

It was apparent that the small differences in

temperatures of storage between modern chain stores had less effect on the shelf-life than that exerted by the original microflora present on shipments from the packing houses. Many packers' samples would have met a suggested standard of 1×10^5 bacteria/gram total count. Almost all packers' samples would have met such a standard had the "pin-point" colonies been eliminated.

INTRODUCTION AND HISTORICAL

In recent years Public Health authorities have become increasingly concerned with meat hygiene. Paradoxically, the modern self-serve meats have introduced an element of risk to the consumer in terms of spoilage and potential food poisoning. This is due partly to the introduction of moist low-salt quick pump-cures and fast smoking and partly to the preslicing of meats, the skinning of wieners and hand packaging after heat processing. In addition, the use of films of low moisture permeability in the interest of avoiding shrinkage with its economic implications and, as well, films of low gas permeability such as Cryovac in order to maintain good colour and sales appeal in cured meats provide an environment apparently favourable to the growth of bacteria. There has been undue faith placed in the ability of refrigeration to suppress the growth of bacteria not only at wholesale and retail levels, but in the home (1, 33, 34, 58).

In contrast, the older high salt slow cure and slow smoking produced a relatively dry stable product which

could be kept for extended periods without refrigeration. Bulk meats kept better than those which had been sliced or comminuted and handled excessively. Psychrophiles were of little significance and surfaces being relatively dry little opportunity was presented for surface contaminants to initiate growth and spoilage (10).

Semi-preserved meats include hams and smoked sausages. Their status may be compared to that of pasteurized milk. Storage life is limited although it may vary with the type of curing, the temperature attained during treatment, the type of wrap and the storage temperature (35).

The great majority of food poisonings and infections reported in recent years have been traced to meats and meat foods (14, 52). For example of 300 cases reported in Great Britain in 1953, 199 were attributed to meats and of these 164 to processed meats (56).

In a more detailed report (61) will be found notes on the expected microflora as it relates to heat processing and on those organisms implicated in spoilage and in food poisonings and infections. Suggested

standards and codes relating to packing house and retail practices are also presented. Previous surveys having a bearing on methods are dealt with more fully than is possible at this time. A more extended bibliography is also incorporated in the earlier report.

The bibliography is not confined to North American publications and it must be noted that standards of meat hygiene in Canada are already among the highest in the world. However this need not deter public health officials from attempting to raise standards to an even higher level. Canada has had two centuries of meat hygiene legislation (14) but so far has not adopted bacteriological standards for processed meats, although maintaining such standards in many other foods. This is largely due to the fact that there is such a variety of meat foods and several standards would be required. Thus problems of time, expense and the number of laboratory workers needed would be multiplied (59). Small differences in technique and procedure used in different laboratories make an arbitrary nation wide standard difficult to apply (37). As a guide in formulating standards for processed meats it may be noted

that the Frozen Food Committee of the Association of Food and Drug Officials of the United States suggested a maximum total count of 1×10^5 colonies per gram and the Canadian government fisheries code suggested a total count not exceeding 2.5×10^5 colonies per gram.

The larger packing houses conform to standards of their own at the time of manufacture but there is often a considerable time lapse before the meat reaches the consumer. Therefore work was undertaken in an attempt to answer the following questions posed by Jepsen (35):

(i) Are the bacterial counts at the moment of sale in conformity with those to be expected from a heat treated product?

(ii) Does the keeping quality permit a reasonable shelf life?

(iii) Are the products in a condition involving risk of food poisoning?

Historical

Previous surveys on frankfurters include the following: Consumers' Union investigators reported 25%

of the 400 packets tested as having high total bacterial counts although coliform counts were low (9). Drake, Evans and Niven (15) found retail total counts on 36 packets from 11 companies ranged from 1 x 10^4/gm to 1 x 10^8/gm. Ogilvy and Ayres (49, 50) and Kraft and Ayres (40) investigated the effect of carbon dioxide within various wraps used on frankfurters.

Previous surveys on ham include the following: Goldenburg, Sheppey and Robson (21) tested 490 canned hams and found 86% to be satisfactory; those which were not, frequently showed the presence of faecal streptococci. Clarenburg (8) and Hobbs (24) also reported faecal streptococci in hams. Barnes, Ingram and Ingram (2) and Barnes (3) found faecal streptococci to be a prevailing contaminant in bacon factories.

Purpose of the Investigation

Some investigators made use of samples from the packers, others tested at retail level; almost all appeared to use random samplings. The need for a comprehensive survey following the packer's meat through

retail channels seemed evident. Use of code-dates would enable us to compare the handling at different packers and retailers. Our aim was to compare samples of the same meat kept under different conditions, e.g. of wrap or temperature and also to compare different meats kept under identical conditions. The survey was designed to include an assessment of the real shelf life in contrast to the optimum and to determine the reliability of results of tests made on single random samples as commonly submitted by health inspectors. Consideration was to be given to the possibility of setting up bacteriological standards for heat processed packaged meats in terms of spoilage organisms and of pathogens. It was hoped that our results might indicate where such standards might best be applied.

GENERAL CONSIDERATIONS

Considerations Influencing the Choice of Meats to be Sampled and their Source.

It was considered necessary to restrict the number of meat types used in the survey to two in order that adequate replication in sampling could be employed. The meats chosen represent two types in very common usage; the comminuted cured smoked sausage and, secondly, sliced cured cooked ham which is unsmoked. Preliminary investigations indicated that various meat loaf products and corned beef would fall, bacteriologically, into a group intermediate between those two types chosen. For practical purposes, one pound packets of wieners were easier to handle and more readily available at all retail outlets than were packets of bologna which is more commonly sold in bulk. Accordingly, wieners were chosen as representing the smoked sausage group. In support of this selection of meat types the following statistics are presented. In 1950 wieners constituted over 40% of the North American sausage production; a total of some

two and one half billion pounds (58). Cooked ham production in North America for 1955 reached some two billion pounds.

Of all meats implicated in food poisonings, cooked ham is the most frequently involved (19). It should be emphasized that most sliced cooked ham is consumed without further cooking thus bacterial counts presented in the Tables of Results represent bacteria actually consumed. In contrast, reported number of bacteria on wieners would be somewhat reduced by cooking before consumption although published cooking instructions often call for too brief a heat exposure to reduce significantly the bacterial populations present.

Two government-inspected packing houses were selected as representing a relatively high standard of quality. These are designated by the letters X and Y. Both packers represent branches of national companies shipping from coast to coast.

Retail samples were collected from branches of modern chain stores operating in Metropolitan Winnipeg. These are designated as A, B, C, etc. During the first

year of the investigation, more than one branch of each Company was used since samples were not always available as required. In the second year, code-dated samples from the packers were placed with one outlet representing each chain in order to permit more adequate replication and experimental control.

Conditions Influencing Methods of Sampling

Packing house laboratories commonly employ surface counts in routine examination of products after heat processing and occasionally make use of internal samples to check on the adequacy of processing methods (23). Since surface and internal microflora often differ in type, it was apparent that a cross-section representing both surface and internal populations was more desirable. Since sliced ham already represents a cross-section of the bulk ham a cut was made across the centre of the packet to ensure access to a more uniform sample. Similarly, wieners were also sliced transversely through the centre to provide a more uniform sample. It should be noted, however, that spoilage of wieners and sliced

ham most frequently occurs first at the extremities of the wieners and the edges of the ham slices. Accordingly, the bacterial counts presented in the Tables probably are lower than would be the case if the ends of packets and edges of slices had been sampled.

During the first year of the investigation each packet was considered as a separate sample. When it became evident that considerable variation in counts existed between packets of a single code-date, the sampling method was changed to allow for blending of four subsample packets into one bulk sample. The bulk sample was then considered as more truly representative of a single code-date from one packer. This blending was done on subsamples from both wholesale and retail level. The blending practice involved collection of eight packets of one code-date; four were left at retail for shelf-life tests after a seven day interval and four were examined immediately. The blending practice ensured a greater statistical reliability of sample (1) since it had been reported by a number of workers that meats, bacteriologically speaking are not homogenous.

Considerations Influencing the Choice of Method of Analysis

The results of previous surveys were examined and consideration was given to methods which could be carried out by one investigator in order to yield as much information as possible. Biochemical tests such as dye reduction, pH determination, indol and H_2S production, register metabolic activity rather than the numbers of microorganisms present (37) and are not satisfactory for assessing a mixed population. Similarly the Most Probable Numbers technique is not readily applicable since the possibility exists of one particular population emerging as the dominant one, to the detrement of statistical reliability (55).

Of the various methods considered, the plate count has the advantage of revealing not only types but numbers (32). The method as commonly employed has inherent in it a high coefficient of variability (46) but it provides a rough estimate of the population alive in the sample, or at least that part of it capable of proliferating in the medium at the temperature chosen (37). Such total counts may be of greater value when used in conjunction with

selective media for specific groups of bacteria (37, 63) though food particles sometimes interfere with selectivity (1).

It is often stated that the count of viable micro-organisms in a food is of little value compared to the identification of the species present. This does not necessarily apply to cooked meat products, for if the meat has been carefully prepared the viable counts should be low (23, 35). Bacteria of low heat tolerance may be assumed to have been introduced by handling after heat processing. Counts on slightly salted cooked meats correlate well with spoilage (35). Thus the quality is related to the action of the microorganisms present and the total count gives an indication of the care taken in preparation and handling of a meat (37).

While it is true that food poisoning bacteria may be present in food showing no obvious signs of spoilage (32) the majority of foods implicated in food poisonings and infections yield viable counts higher than 1×10^6 colonies per gram (14, 24). Conversely the majority of foodstuffs not associated with poisonings or infections

yield counts of less than 1 x 10^4 per gram at 37° C incubation (24).

Adequate facilities were not available in the laboratory for inclusion of anaerobic counts (21).

In view of the foregoing considerations it was decided that the main body of the survey should consist of total counts on plates incubated aerobically at three temperatures. A number of media selective for Salmonellae, coliforms, coagulase positive staphylococci and enterococci were also to be employed. Mould counts and lipolytic counts were to be included. In addition selective broths were to be used as qualitative indicators. In each case population estimates were to be based on the average count of five replicates per dilution.

Considerations Influencing the Choice of Media and Incubation Conditions.

At all stages prior to consumption of a meal, meat is liable to contamination by bacteria with widely varying properties. Their number and type may be influenced initially by the physiological condition of the animal at the time of

slaughter; a high glycogen level insures a satisfactorily low pH, together with the desired Eh changes affecting the microflora introduced at the time of killing.

Grinding spreads surface contamination through the meat and influences the proportion of fat to connective tissue and the amount of air in the meat. Many gram positive bacteria having a lipophylic surface, can survive heating even at 78° C for three hours in the presence of fat (20, 27).

Additives may introduce their bacterial flora and affect the growth of bacteria already present in the meat. The addition of carbohydrates may replace a proteolytic flora with saccharolytic yeasts and lactobacilli. Curing may favour salt tolerant yeasts, lactobacilli, micrococci and enterococci (10, 11, 44, 45). These may multiply during the initial stages of smoking (45). Other bacteria may be introduced at the time of wrapping, and the oxygen reduction potential of the packet and its storage temperature may also influence the expected population.

It is therefore necessary to investigate the activities of a large number of microorganisms which may differ widely both in their growth requirements and in

the effects produced by such growth (23). Metabolic changes caused by heat, cold or curing may make the surviving population more fastidious nutritionally than the population found on fresh meats (1). Thus the pH, salinity and composition of the medium may have to be modified to suit the flora. Preliminary experiments indicated that numbers of bacteria recovered were greater on Tryptone Glucose Yeast agar than on nutrient agar. Therefore Bacto Plate Count Agar was chosen for enumeration of total counts. In the interests of maintaining quantitative results no enrichment media were used (14).

Incubation temperatures were also considered in the light of the previous history of the meat and the expected population (23). A sufficiently wide range of temperature for incubation was available in the laboratory. Seventy two hour incubation periods were selected for total counts at 25° C and 37° C. The psychrophilic count was made at ten days (63). Selective media were used according to instructions.

With the exception of plates containing Enterococcus agar (57) all plates were incubated in an inverted

position.

In summation, media and time-temperature relationships for incubation are presented in Table I.

Considerations Influencing the Choice of Diluent

Obligate halophiles are no longer common in commercial brines. Accordingly, the use of sodium chloride in dilution blanks for its osmotic effect is unnecessary. Its presence in higher concentrations, moreover, may even depress the development of some species present.

Dilution blanks were adjusted to pH 7.0 with Sorenson's phosphate buffer to provide conditions considered as approaching a pH optimum for the majority of bacterial species likely to be present. Preliminary experiments using a 10 g sample in one litre of diluent were unsatisfactory. Addition of various surface tension depressants to the diluent in the hope of obtaining a more homogenous suspension produced erratic results. After a number of trials a diluent volume of 99 ml was selected because this gave a satisfactorily uniform slurry.

EXPERIMENTAL

Methods

Except where indicated all packets for sampling were removed to the laboratory for examination on the day of collection. The only exception to this practice occurred for experiments in Series II. In this case wiener packets were sampled at each collection site by aseptic removal of the wiener sample to a sterile polyethylene bag. The remaining wieners in the packet were carefully rewrapped and left at the storage site for subsequent sampling and examination at regular intervals to determine the effect of these particular storage conditions on the development of microflora.

Sampling Techniques

In general, 10 gm of meat were removed aseptically to constitute a sample. In the case of wieners, this was usually done by combining thin cross sections of several wieners from one packet. In the case of ham, the sample

was obtained by cutting through several slices to ensure a more representative aliquot. Exceptions to this practice occurred in experiments in Series II and Series IV. In the former instance, a 10 gram sample was removed from the centre of each wiener on the day of collection; while in Series IV 100 gram samples were taken as representative of each packet. In this instance, four such samples were placed in a sterile Waring blendor and mascerated without added diluent. From the resulting puree a 10 gram sample was transferred to a second sterile blendor to which 99 ml of diluent were added. This constituted the first 1:10 dilution for experiments in Series IV.

Blending

Blending of all samples was done for three minutes at high speed (43). At the conclusion of the blending period air bubbles were allowed to subside to ensure accuracy of pipetting volumes. All transfers by pipette were carried out while the blended sample was still homogeneous.

Dilutions

Packers' samples were generally plated at dilutions of 1:100 and 1:1000 for total plate count, and at 1:10 where selective media were used. These dilutions for plating were chosen to permit a counting range up to 250,000/gram as outlined in the Canadian Government Fisheries Code.

Plating of samples from retailers took place after the packers' samples bearing the same production code-date had been examined and counted. Unless numerous pin-point colonies had been noted in the packers' sample it was customary to use dilutions ten to one hundred times higher for the retail samples. Where large numbers of pin-point colonies had been noted in the packers' samples dilutions for retail were 1:1000,000 and 1:10,000,000. It may be pointed out that in some cases even plates from these high dilutions were so crowded with pin-point colonies as to permit only a rough estimate of the number.

Plating of all samples was done in quintuplicate at each of two dilutions for each temperature of incubation.

Reading of Plates

Wherever possible only those plates bearing between 30 and 300 colonies were counted using a Quebec Colony Counter. As each colony was counted its position was checked off with a wax pencil to avoid counting errors. Results were reported as an average of counts of five plates per dilution. Occasionally, for unknown reasons, the number of colonies on one of the five plates was significantly different as measured by the Chi square test (18) from those of the other four. In such rare cases the variant plate was rejected as an unreliable statistic.

Each colony was counted as one even when it consisted of a spreader type or a pin-point. In a few instances the count of pin-point colonies was complicated by the presence of minute meat particles in suspension in the agar medium. It became possible, however, with increasing experience to distinguish pin-points from meat particles. At high dilutions each pin-point appears as a separate colony. With prolonged incubation some of these attain the size of a pin-head while others remain minute. Ingram et al (30) described similar colonies from brine samples.

Pin-points and spreader colonies have been blamed for abnormal variations in count (18) as have fungi (31). In this survey the numbers of pin-points in each of the five plates of a dilution set when present seemed to be consistent. In those cases where numbers of pin-point colonies were greater than 300 per plate, counting was done by sectoring the plate and counting each of at least two sectors. The average of sector counts times the appropriate sector factor gave the final count for that plate.

Method of Presentation of Results

The presence of numerous pin-point colonies posed a problem in presentation of results which, to a certain extent, obviated the use of conventional practices. For example, graphical presentation is quite impractical when one set of data represents information from a group of plates with only a few hundred colonies; while the next set of data was derived from plates bearing many thousands of pin-point colonies.

Accordingly, an index was devised in order to facilitate comparisons (38, 55). The ratio of final population to initial population as a numerical indication of population change in storage was suitable providing there was no more than one variable, i.e. time. An example follows: The increase in microflora population ("growth change") in one type of meat of given code-date could be determined for each of several retail storage sites. A ratio of final population to initial population thus would indicate the effect of such storage conditions. Any increase in populations would yield a ratio value greater than unity. A ratio value of less than unity

indicates a decrease in population from the level present when the meat was received from the packer.

With few exceptions the counts on tellurite glycine and desoxycholate media were too low to be reported with any accuracy. Similarly, mold counts were low and are not included in the report. As indicated in Table I, counts of lipolytic bacteria were attempted on each sample throughout the investigation. The interference effects of the lipolytic spreading colonies was such that the inclusion of these data was not considered advisable. Conventional methods for enumerating lipolytic microflora are not readily applicable to heterogeneous populations from processed meats.

Results of tests on various broths are not included since these were qualitative rather than quantitative. Thioglycollate tubes were generally positive. Lactose bile green broth tubes were generally negative confirming the results obtained on desoxycholate agar. Enterococcus presumptive broth proved unsatisfactory because the pH of the meat in low dilution was often sufficient to produce a color change in the medium immediately on addition of the sample inoculum. When higher dilutions

were employed in an attempt to avoid the pH colour effect, negative results were obtained. In this respect it should be noted that S. faecalis growth is favoured in this medium as compared to S. faecium (38) and the latter species is the one encountered most frequently in our samples.

Table I. Summary of Media Employed and Time and Temperature of Incubation for the Various Populations Estimated.

Medium	Incubation Temperature	Time in days	Population estimated
A. Semi-solid Media			
Std. Plate Count Agar (Difco)	10° C	10	total aerobic count
	25° C	3	total aerobic count
	37° C	3	total aerobic count
Lipolytic Medium (36, 53)	25° C	3	lipolytic bacteria
Martins Rose Bengal Agar (42)	25° C	3	moulds
Tellurite Glycine Agar (B.B.L.)	37° C	1	group D staphylococci
Desoxycholate Agar (B.B.L.)	37° C	1	coliforms, *Salmonella* spp.

Table I (cont'd)

Medium	Temperature	Time in days	Population Estimated
Enterococcus Agar (57)	10° C	10	faecal streptococci
	25° C	3	faecal streptococci
	37° C	3	faecal streptococci
B. Broth Media			
Enterococcus Broth (Difco)	45° C	2	faecal streptococci
Brilliant Green Bile 2% (Difco)	37° C	1	coliforms
Thioglycollate Broth (Difco)	37° C	2	anaerobes (qualitatively)

RESULTS AND DISCUSSION

Results tabulated in this report are confined to those based on samples bearing the same code date at packing house and retail level. Accordingly, results of Series I are not included; these were based on random samples and did not provide sufficient information on which to assess handling at packing house or retailer. Series I demonstrated the tremendous range of counts possible in meats of the same age. It was also evident that bacterial populations increased during prolonged cold storage, to such an extent that tables of results in an earlier report had to be presented reduced by 10^6; this was particularly true of psychrophilic counts. Products from Packer X tended to carry a heavier bacterial load than those from Packer Y.

Series II

Series II was designed to reduce to a minimum the variation in counts by sampling repeatedly at intervals from one packet at each retailer. Once packets had been

opened and the initial sample taken, they were carefully rewrapped and placed in sterile polyethylene bags. This was to avoid subsequent contamination and inadvertent sale of the packet by the retailer. Any alteration in environment was the same for all samples.

Results and analysis of Series II, Expt. 1 are presented in Tables II-1 and II-2 respectively. The initial retail count on Y wieners at retailer A was greater at all three incubation temperatures than the initial retail count of the sample at E. After one week's storage the count at A had decreased more than the count at E. While counts at A dropped at all three incubation temperatures, those at E increased for 25° C and 37° C incubation. The counts at U (control at 3° C at University) increased markedly at 10° C and 25°C while dropping at 37° C incubation.

Results and analysis of Series II, Expt. 2 are presented in Table II-3. There was considerable growth at all incubation temperatures, particularly at 10° C. Table II-4 indicates that wieners of different code dates from one packer, held at the same retailer may bear very

different populations. Both samples bore similar numbers at six days on plates incubated at 10° C. After a further seven day storage at retail there was a considerable difference in counts, particularly on plates incubated at 10° C.

Results and analysis of Series II, Expt. 3 are presented in Tables II-5 and II-6. The population on wieners from Packer X held at U (control) declined at 37° C incubation temperature whereas the counts on wieners held at B increased at 37° C incubation. Counts on sample at B increased faster over a sixteen day period than did counts on the sample at U despite the fact that the sample at B had a smaller population initially.

Results and analysis of Series II, Expt. 4 are shown in Tables II-7 and II-8. There was a decrease in numbers during the first two days of storage at both sites. The population was initially lower on wieners at A than at C and remained so after five days, but increased rapidly between the fifth and nineteenth day. Unfortunately the sample at C was discarded by the retailer before a final test could be made.

From this series it was possible to compare the

retailers for each time interval they had in common and for each temperature of incubation provided that the wieners were of the same code date. However all retail storage temperatures were less than 4° C (Table VIII) and emphasis must therefore be placed on the numbers and species of bacteria present when the packets left the wrapping line at the packing house. Variations in temperature tolerance of different species would account for differences in growth, some preferring 10° C to 37° C.

In general the results of Series II show that counts from Packer Y are lower and show less variation than counts on wieners from Packer X. It appears that Y samples were generally free from pin-point type colonies and the population was not likely to increase substantially even with further holding. The sample in Expt. 2 (Table II-3) shows that this is not invariable. Wieners from Packer X frequently had great numbers of minute colonies which continued to grow under refrigeration.

Series III

Experiments in Series III made use of quadruple

sampling at the packers in an attempt to discover how much variation in counts could be attributed to handling at the packing house. Each of four retailers was represented by one packet bearing the same code date. The populations of the retail packets were to be compared to each other and to the initial counts on packets of the same code date from the packing house.

Unfortunately the variation in results from the four packer's samples (Table VI) was much greater than had been anticipated. It became necessary to compare individual retail counts with a mean (M-1) of the four initial packing house samples, which gives only a rough comparison of the effect of retail handling. In order to assess the overall population of meats of one code date the mean of retail counts (M_8) is also included; for Y samples (Table III-2) whose counts showed little variation, the ratio M_8/M_1 may provide a reasonably accurate assessment of quality for any packets of that code date. However for X samples where there is great variation on counts (Tables III-4 and III-6) the seven day mean ratio can only serve as a rough guide to the quality of a given packet.

Series III, Expt. 1. Results and analysis are presented in Table III-1 and Table III-2. Counts on sample at retailer H were higher than those at D for incubation temperatures of 25° C and 37° C. Meats at both retailers showed an increase in populations at all three temperatures of incubation.

Series III, Expt. 2. Results and analysis are presented in Tables III-3 and III-4. Retailer D had the sample bearing the largest numbers. This was not likely due to retail carelessness since the count on the sample at D in the previous experiment was low. Rather it illustrates the effect exerted by differences in species present originally. If pin-point type colonies are present initially in a refrigerated sample the retailer can do nothing to reduce their numbers. The sample at F was apparently free from pin-points, consequently the population decreased at all three temperatures of incubation.

The most obvious trend evident in Series III was the tremendous variation in counts on packets of one code date. Again the population on Y wieners in cello was low as compared to X wieners in cello (Tables III-1 and III-3).

Furthermore counts for X wieners in Cryovac (Table III-5) were very much greater than for X wieners in cello, even allowing for variation in meats of different code dates. This would indicate that the pin-point population prefers the micro-aerophilic environment of Cryovac which is relatively impermeable to gases and moisture compared to cello.

Series IV

Series IV was designed to minimize the effect of variation in packets of one code date revealed in Series III. Bulked samples made up of meat from four packets were taken at both packer and retailer. All eight packets were taken together from the wrapping line at the packing house. Four were tested the same day and four were left with one retailer for six days.

Storage of samples in the refrigerated sales counters at retail was impossible, because of the danger of the samples being sold inadvertently. Therefore the samples were left in the refrigerated space beneath the counter, or in the walk-in refrigerator. Retail

temperatures were noted and compared to those at which wrapped meats were displayed for sale (Table IX).

Results and analysis of experiments 1-8 on Y wieners, are presented in Tables IV-1 to IV-4. One sample at each retailer showed an overall decrease in population at all three temperatures of incubation (Expts. 2, 4, 6 and 7). Of the other samples, all four increased at 10° C incubation, two showed a slight drop at 37° C incubation (Expts. 3 and 8) and two a slight increase at 37° C incubation (Expts. 1 and 5).

Results and analysis of experiments 9-16 on X wieners are presented in Tables IV-5 to IV-8. Only two retail samples showed a marked decrease in counts; both were at G. For the other samples the increase at 37° C incubation generally appeared to be smaller than that at 10° C. Exceptions were one sample at retailer F and one at H (Table IV-8, Expts. 12 and 14).

Ratios based on the average counts of two samples for each packer at each retailer, showed that for X wieners retailers F and H appeared to give a higher count than retailers N and G, (Table IV-8). In contrast

retailers N and G appeared to give higher counts for Y wieners than did retailers F and H (Table IV-4). Again this is probably of little significance, the temperatures of storage at all four retailers being between 35° F and 38° F, all of which were below 4° C.

For meats of different code dates, retail comparisons can be no more than approximate, when consideration is given to the different bacterial flora coming from the packers on different days. However, comparisons of handling at the packing house may be made by comparing counts at one retailer where meats are held under identical conditions. Packer handling may also be assessed by comparing the average counts for all the retailers, on wieners from the two packers.

Ratios based on counts at individual retailers show a marked difference between the packers; counts on X wieners (Table IV-8) being much higher than counts on Y wieners (Table IV-4). The one exception being the samples at retailer G.

Ratios based on average counts from all four retailers (Tables IV-3 and IV-7) show the same tendency

but the difference is less marked.

Individual retail values are based on 32 packets whereas the average retail values are based on 128 packets. The use of averages tends to even out differences in counts, e.g. the wide range of counts on X wieners (Tables IV-6) is not shown up by the averages.

In general the increase in population for X wieners is greater at 10° C than 25° C and 37° C incubation. An increase in count is generally evident at all three temperatures of incubation if pin-points are present in Cryovac wrapped wieners (Table IV-7). In contrast the population on Y cello wrapped wieners (Table IV-3) declines at 25° C and 37° C incubation. The increase on plates incubated at 10° C is less than half as great for Y cello wrapped wieners as for X wieners wrapped in Cryovac. Different species are probably involved.

Tables IV-11 includes counts on Enterococcus agar which was used during the second half of Series IV. It is evident that high total counts (Tables IV-1 and IV-5) are paralleled by high Enterococcus counts (Table IV-11). Enterococci are not invariably present on X wieners

(Table IV-11, Expt. 16), but they are found much more frequently than they are on Y wieners.

Table IV-3 indicates little difference in the ratios for groups 1 and 2 into which Y wieners were divided in order to assess the effect of holding Group 1 for four days before wrapping. The difference would likely have been greater had Enterococci been present in numbers. All Packer X samples were the same age when wrapped and collected, so there was no opportunity to ascertain whether the ratios for X wieners cooked at different times prior to wrapping would have been the same.

In general the results of Series IV show that counts on X wieners are higher and vary more than counts on Y wieners (Tables IV-2 and IV-6). This may relate to the low proportion of Enterococci present in Y wiener samples as compared to X wiener samples.

Reasons for this difference may include the following:

(1) Difference in the diameter of wieners would influence the effective heat penetration during smoking and cooking; X packs nine wieners to a pound in contrast

to the twelve packed by Y.

(2) Difference in the oxygen/reduction potential within the wrapping film. X has recently changed from Cello wrap to Cryovac wrap for wieners; Y still uses the Cello wrap. Cryovac is relatively impermeable to gases and moisture and appears to encourage the growth of microaerophiles such as Enterococci. The Enterococci increase relatively slowly when meat is wrapped in the more permeable cello.

(3) Use of ascorbate by X would add further to the reducing condition.

Series V

Series III had been designed to include ham but it was impossible to collect the required packets at retail level. The initial counts (Table VII) for ham showed a great variation as did counts on many packets of wieners. Therefore it seemed important to ascertain the effect of a week's storage on ham, under the same condition as wieners. Series V was designed to parallel Series IV, with ham under investigation instead of wieners.

Time allowed for only one set of results on ham for each retailer.

Results and analysis of Series V are presented in Table V-1, et seq. Comparison of Tables V-1 and V-5 indicates that there is less overall difference apparent between populations on ham from Packers X and Y, than was evident on wieners.

Ham from both packers is subject to the appearance of pin-point colonies and when these are present counts on X ham are higher than counts on Y ham. Counts on ham from both packers are either very high if pin-points are present, or very low if they are not; intermediate numbers were few.

Experiments 1 and 5 show that on long-term storage, counts are in the range of 20-200 million which is too high for a product which is consumed without further cooking. Counts at retailer N were ten times higher for X ham than for Y ham at 10° C incubation.

However, it is apparent that only a small percentage of the pin-point colonies from ham are Enterococci. Y samples incubated at 37° C (Table V-1)

bore a great number of pin-points only 0.1% of which were confirmed as Enterococci (Table V-11). The percentage of Enterococci for X ham at retailer N (Tables V-5 and V-11) was even lower.

In general retailers N and G appeared to favour higher counts than retailers F and H on ham from both packers (Tables V-4 and V-8). This is probably due to chance distribution of pin-points rather than to retail handling, little variation in retail storage temperatures being noted.

Again it is evident that ratios comparing ham from packers X and Y are higher when based on counts at individual retailers (Table V-9) than when counts from all retailers are added (Table V-10) and averaged.

At 10° C the ratio X/Y is lower for ham than for wieners but at 25° C and 37° C it is considerably higher than it was for wieners. Increase in numbers was apparent on ham samples from both packers at all three incubation temperatures, indicating the presence of a more varied bacterial population on ham than on wieners.

The interval between cooking and wrapping tends to

be longer for ham than for wieners; some hams being kept up to ten days before being sliced and wrapped. This prolonged cold storage allows for considerable bacterial growth before the meat is shipped.

Table VI-1 and VI-2 present the counts on one day wieners subsamples and shows the variation in numbers possible, within four packets bearing the same code date. It appears that for both packers, the winter count seems to be slightly lower than the summer count. There is little difference in counts on cello wrapped samples from both packers taken in the winter. However the count on Cryovac wrapped wieners from packer X are much higher than on Cello wrapped wieners from packer Y taken in the summer. In general the higher the count, the greater the variation.

When consideration is given to the fact that the tables present counts reduced by 10^3, the variation in numbers is sufficient to cast doubt on the value of random sampling as a means of quality control.

Table VII presents the counts on ham one day after slicing and wrapping. The same argument obtains here as

as for wieners; variation is sufficient to make random sampling doubtful.

Variation between packets of wieners of one code date (Tables VI-1 and VI-2) probably relates not only to the homogeneity of the meat but to the efficacy of smoking. Points of contact between the wieners and the stick and between each other may result in lack of smoking uniformity. The temperature of the wieners on entering the smokehouse and the height of the wiener on the stick may influence the internal temperature attained, as does the diameter of the wiener itself. Further variation may be introduced during tempering to facilitate skinning and in hand packaging. The efficiency of the smokehouse is of paramount importance because it is here that any bacteria present should be destroyed. If the heat is inadequate to reduce the numbers of bacteria significantly, smoking may serve to incubate any heat resistant bacteria present. Many of these are capable of growing under subsequent refrigeration conditions. Others will grow well at 50° F, the temperature commonly employed for tempering and wrapping.

Variation between packets of ham of one code date (Table VII-11) probably results from slicing and hand wrapping practices. The slices at each end of the oblong block receive greater treatment than do those in the centre and might be expected to carry a smaller bacterial load. Ham is not generally sliced the day it is cooked but may be sliced to order at the packing house up to ten days after cooking. Were ham shipped sooner the bacterial population would have less time to increase. This is the policy behind the design of new packing houses with minimum cold storage facilities (20).

It has been emphasized that the main responsibility for high counts must rest with the packer. The retailer's chief responsibility is to see that the packets are adequately refrigerated and sold before they become overage. From Table IX it may be observed that during the second year survey all four city retailers kept both wieners and ham for periods exceeding seven days despite their avowed company policies to the contrary. Code dates of displayed meats were checked over a period of four months every time samples were deposited or collected.

This was done in an effort to determine whether the time interval chosen for testing was realistic. Each figure represents one or more packets of that age present in the sales counter. These lapses were not the results of carelessness on one or two days but were fairly consistent. A minimum of six visits was made to each retailer. These examinations indicate that more care should be taken in checking code dates regularly (9); meats should be sold in order of receipt from the packer (39, 62). It cannot be stressed too strongly that the terms "cured" and "vacuum packed" do not infer sterility or even stability. Ideally the consumer should be able to check the code dates; this would help the health inspector and encourage retailers to compete in rapid turnover. It is to be expected that retail stores in outlying districts would tend to have stocks even older than those in the city.

Should retail investigation ever be established, the method used in Series II provides a fairer assessment of holding conditions than does random sampling, which is of little value unless the search is for specific pathogens. Removal of part of a packet to the laboratory

for testing while leaving the balance with the retailer for testing later, provides two sets of results economically and with variation reduced to the minimum. A comparison of counts from the tests would indicate whether the population was increasing or decreasing.

The same method might well be employed in comparing the effect of various storage conditions within one retail outlet; packets could be sampled and replaced above the load line, on bargain tables or under proper refrigeration for a given interval and subsequently retested.

Investigation of Pin-point Colonies

Throughout the survey it was evident that despite every attempt to avoid errors in sampling and technique, the interpretation of results was often hampered by the appearance of very large numbers of minute colonies. Since these were not invariably present their appearance made it difficult to compare storage sites. Accordingly although the survey did not include a breakdown of total count into species, it seemed advisable to determine

whether the pin-point colonies were likely to influence the shelf life or wholesomeness of the meat.

The majority of the pin-points grew as sub-surface colonies on aerobic plates, hence transfer by needle was very difficult (30). Even those colonies on the surface did not subculture readily by the usual methods. Therefore enrichment cultures for use as inocula in diagnostic tests were prepared by removing agar containing pin-point colonies to thioglycollate broth (13).

Slides for microscopic examination were prepared by imprinting colonial growth on a cover-slip by pressing it firmly on to the exposed colony surface. All the colonies appeared to consist of Gram positive cocci in pairs and chains. These were apparently faecal Streptococci. Tests for tolerance to temperature, pH and high salt concentration and biochemical tests appeared to confirm our opinion.
A duplicate set of diagnostic tests was prepared for incubation under a CO_2 atmosphere in a Brewer jar. Growth in these tubes was more rapid and results were available 24 hours earlier than for those tests incubated under a normal atmosphere. Under aerobic conditions growth was

very good in thioglycollate broth to which $CaCO_3$ had been added, good in trypticase soy broth and brain heart infusion broth but poor in Enterococcus presumptive broth (Difco). Tween 80 was added to dilution blanks during the course of earlier experiments in an attempt to form homogenous suspensions of fatty meats. Pin-point growth was apparently stimulated by addition of Tween 80 (12, 13) to plates incubated without added CO_2.

There appeared to be sufficient justification for introducing the M-Enterococcus agar of Slanetz and Bartley into the routine plating (Tables IV-11 and V-11). As well, incubation of plates at 45° C was resumed. Millipore filters and pour plates were used but although counts correlated well, there appeared to be no advantage in using filters in this case. Frequently counting on filter surfaces was complicated by the development of numerous colonies. One millipore filter was used at high dilution however, as a routine check. It was found to be advantageous to place the millipore filter disc face down on the bottom of the Petri dish before adding the agar. This served to reduce the oxygen tension in the vicinity

of the colonies. Similarly for pour plates an extra layer of agar was added over the inoculated medium when it had set.

Results of this pilot experiment are included in Tables IV-11 and V-11. It was evident that in some cases the numbers on plate count agar were far in excess of those appearing on M-Enterococcus agar. In others, counts correlated well on both media. Contrary to expectation, counts were generally reduced on plates incubated at 45° C. This reduction in numbers could not be attributed to dehydration because other plates in the same incubator showed good growth. Therefore it is apparent that not all pin-point colonies are faecal Streptococci. It seems probable that among the pin-point colony formers may be found S. lactis, Leuconostoc spp. and Pediococcus spp. (10, 29, 38). Oddly enough there was better agreement between counts on plate count agar and Enterococcus agar for wiener samples than for ham samples, although the wieners might well be expected to carry a load of Lactobacilli present in the dried milk added.

It is regrettable in view of the introduction of

wrapping films relatively impermeable to gas and to moisture vapour (22) that an anaerobic survey could not be carried out. Since heat processing is in many cases insufficient to kill faecal Streptococci, it is possible that spores of anaerobes could also survive, both in ham and wieners. This question must await investigation.

Due to the necessity for the continuation of routine sampling and testing, time was not available to pursue the identification of those pin-point colonies which were not Enterococci. Further research is necessary on this point. The fact remains that the sale of meats bearing a heavy load of bacteria, regardless of species, should be discouraged. These may represent species surviving the cure and heat processing, or those introduced subsequently in handling. In either case the packer must bear most of the responsibility since the packets are sealed before they leave the packing house, and are not liable to further contamination at retail.

Many of the samples tested met the standard consisting of 10^5/gm total count, less than 10 coliforms

and less than 100 Group D Staphylococci per gram as suggested by various workers. Most samples would have met this standard had pin-point colonies been eliminated. Both packers have succeeded in eliminating them from some packets of ham, and Packer Y has managed to do so in most packets of wieners. Therefore it should be possible to approach a standard of 10/gm for Enterococci in processed meats.

Generally speaking the packing houses already have their own standards for various catagories of meats. The results from tests are not usually available however, in time to stop shipment of meat which shows a high bacterial population and which is reported as "unsatisfactory" (59). Therefore in lieu of a hard and fast bacteriological standard, it seems that a code of handling would be easier to enforce. This should insure adequate heating and cooling, use of the correct wrap and minimum inventories for quick turnover at both packer and retail levels.

SUMMARY OF DISCUSSION OF EXPERIMENTAL WORK

Results of experimental work in Series I to Series V include the following general trends:

(1) Counts tended to be higher and to vary more on meat products from Packer X than on those from Packer Y.

(2) In general psychrophilic counts increased with time of refrigerated storage while mesophilic counts showed a corresponding decline. This tendency was more marked with wieners than with ham and appeared to correlate with the presence of Enterococci (faecal Streptococci).

(3) Pin-point colonies developing on plates from wiener samples were predominantly Enterococci. Few pin-point colonies developing from ham samples were confirmed as Enterococci.

(4) Wiener samples wrapped in Cryovac developed larger bacterial populations than did samples wrapped in cello. This was probably due to the microaerophilic nature of the Enterococci.

(5) Variation in both population and species was apparent in packets of one meat bearing one code date

from one packer.

With these trends in mind it is possible to attempt to answer the three questions posed at the outset.

(1) With the exception of the samples harbouring Enterococci, the initial counts are in conformity with a heat treated product, bearing in mind the fact that these meats are hand packaged after heating.

(2) With the exception of the samples harbouring Enterococci, the bacterial counts might permit a reasonable shelf life, i.e. one week at retail. Longer retail storage particularly of sliced cooked ham, is not advisable.

(3) The products do not appear to be in danger of causing food poisoning by Staphylococci or Salmonella spp. The presence of Enterococci does however, serve as a warning that Clostridium spp. may be present. Some Enterococcal strains may cause food poisoning, particularly if the bacteria are present in large numbers.

GUIDE TO INTERPRETATION OF TABLES FOR SERIES II

Series II involved repeated sampling of weiners from single packets and replacement of packet for further retail storage between samplings.

Results of each experiment are presented as total counts reduced by 10^3 for each temperature of incubation.

In order to facilitate tabulation the packers are labelled X and Y; the retailers are labelled A, B, C, etc. The letter "U" denotes samples held in our laboratory cold room at 3° C. Addition of a subscript numeral to the letter indicates the age in days of the sample from that retailer. e.g. A_4, A_6, A_{13} would denote samples held by retailer A for four, six and 13 days respectively.

Analyses of results are presented in the form of ratios of total counts. These may be used to compare populations at different times at one site. For example, if the ratio of final count/initial count is greater than unity, an increase in population has occurred; if less than unity there has been a decline in numbers.

Ratios may also be used to compare growth at different sites provided there is no more than one

variable. e.g. for a stated time interval if ratio total count at A/total count at B is more than unity it follows that sample held at A has a higher population than that held at B.

Use of Ratio Table involved placing the count from the horizontal listing over that on the vertical. For example, to compare the psychrophilic count on samples held at U for four and thirteen days in the first experiment: At 10^{o} C the figure for U_{13} was 7.1 while for U_{4} it was 0.1. Solving the ratio 7.1/0.1 yields a value of 71.0 indicating an increase in population by a factor of 71.

Similarly comparison of A_{6} with E_{6} gives 9/1. Thus sample at A has nine times the population of that at E at six days.

Series II

Table II-1. Packer Y Cello Wrap Weiners at Retailers A. E. U. Total colony counts x 10^3 based on repeated sampling at intervals from one packet at each retailer. All packets bore the same code-date.

Incubation temperature	Total colony counts x 10^3 Retail site and age in days of sample						
	U_4	U_6	U_{13}	A_6	A_{13}	E_6	E_{13}
10° C	0.1	0.5	7.1	9.0	8.6	1.0	0.3
25° C	1.0	1.5	5.8	10.0	4.2	1.6	1.9
37° C	3.0	1.4	0.7	2.5	1.0	1.3	2.4

Table II-2. Comparative growth ratios based on data from Table II-1.

		Ratios					
10° C	Sample Site and age	U_6	U_{13}	A_6	A_{13}	E_6	E_{13}
	U_4	5.0	71.0	-	-	-	-
	U_6	-	14.2	1.8	-	2.0	-
	U_{13}	-	-	-	1.21	-	0.3
	A_6	-	-	-	0.96	0.11	-
	E_6	-	-	9.0	-	-	0.3
	E_{13}	-	-	-	4.3	-	-
25° C	U_4	1.5	58.0	-	-	-	-
	U_6	-	3.8	6.6	-	1.1	-
	U_{13}	-	-	-	0.7	-	0.3
	A_6	-	-	-	0.4	0.2	-
	E_6	-	-	6.2	-	-	1.2
	E_{13}	-	-	-	2.2	-	-

Table II-2 (cont).

37° C	Sample Site and age	Ratios					
		U_6	U_{13}	A_6	A_{13}	E_6	E_{13}
	U_4	0.5	0.2	-	-	-	-
	U_6	-	0.5	1.8	-	0.9	-
	U_{13}	-	-	-	1.4	-	3.4
	A_6	-	-	-	0.4	0.5	-
	E_6	-	-	1.9	-	-	1.8
	E_{13}	-	-	-	0.4	-	-

Table II-3. Packer Y Cello Wrap Weiners at Retailer E. Total colony counts x 10^3 based on repeated sampling from single packet.

	Total colony counts x 10^3		
Incubation temperature	Retail site and age of sample		Growth ratio at 7 days final count/initial count
	E_7	E_{14}	
10° C	1.6	86.0	55.0
25° C	60.0	89.0	1.5
37° C	1.3	17.1	13.4

Table II-4. A comparison of seven-day ratios on Y weiners of two different code-dates held at same retailer E. Data from tables II-2 and II-3.

Incubation temperature	Ratios
10° C	55.0/0.3
25° C	1.5/1.2
37° C	13.5/1.8

Table II-5. Packer X Cello Wrap Wieners at Retailers B and U. Total colony counts x 10^3 based on repeated sampling at intervals from one packet at each retailer. All packets bore the same code-date.

Incubation temperature	Total colony counts x 10^3 Retail site and age in days of sample					
	U_4	U_6	U_{22}	B_6	B_8	B_{22}
10° C	0.1	6.0	2000.0	0.7	2.0	1500.0
25° C	15.5	82.0	12000.0	5.5	3.5	2400.0
37° C	110.0	110.0	75.0	4.5	2.0	1700.0

Table II-6. Comparative growth ratios based on data from Table II-5.

		Ratios				
10° C	Sample site and age	U_6	U_{22}	B_6	B_8	B_{22}
	U_4	60.0	-	-	-	-
	U_6	-	333.0	0.1	-	-
	U_{22}	-	-	-	-	0.7
	B_6	-	-	-	2.9	-
	B_8	-	-	-	-	750.0
25° C	U_4	5.3	-	-	-	-
	U_6	-	146.0	0.7	-	-
	U_{22}	-	-	-	-	0.5
	B_6	-	-	-	6.4	-
	B_8	-	-	-	-	700.0
37° C	U_4	0.0	-	-	-	-
	U_6	-	0.6	<0.1	-	-
	U_{22}	-	-	-	-	<0.1
	B_6	-	-	-	45.0	-
	B_8	-	-	-	-	85.0

Table II-7. Packer X Cello Wrap Weiners at Retailers A and C. Total colony counts x 10^3 based on repeated sampling at intervals from one packet at each retailer. All packets bore the same code-date.

Incubation temperature	Total colony counts x 10^3 Retail site and age in days of sample				
	A_3	A_5	A_{19}	C_3	C_5
10° c	25.0	8.0	80000.0	130.0	20.0
25° c	95.0	8.0	24000.0	200.0	50.0
37° c	75.0	50.0	140.0	200.0	45.0

Table II-8. Comparative growth ratios based on data from Table II-7.

		Ratios				
10° C	Sample site and age	A_3	A_5	A_{19}	C_3	C_5
	A_3	-	-	-	5.2	-
	A_5	-	-	10000.0	-	-
	C_3	-	-	-	-	0.2
	C_5	-	0.4	-	-	-
25° C	A_3	-	-	-	2.1	-
	A_5	-	-	3000.0	-	-
	C_3	0.5	-	-	-	0.3
	C_5	-	0.2	-	-	-
37° C	A_3	-	-	-	2.6	-
	A_5	-	-	2.5	-	-
	C_3	0.4	-	-	-	0.2
	C_5	-	1.1	-	-	-

Series III

Table III-1. Packer Y Cello Wrap Weiners at Retailers D and H. Total colony counts x 10^3 based on four day-old subsamples from Packer Y. The four counts on Packers subsamples are averaged and reported as the mean (M_1) for initial count. One subsample at each retailer was taken seven days later; reported as D_8 and H_8 respectively. The average of both retail counts is reported as M_8 for comparative purposes.

	Total colony counts x 10^3			
Incubation temperature	Sample site and age of sample in days			
	M_1	M_8	D_8	H_8
10° c	0.6	1.6	1.9	1.3
25° c	1.8	9.0	3.0	15.0
37° c	1.4	7.4	1.8	13.0

Table III-2. Comparative growth ratios based on data from Table III-1.

		Ratios		
10° C	Sample site and age	M_8	D_8	H_8
	M_1	2.6	3.2	2.2
	M_8	-	1.2	0.8
	D_8	-	-	0.7
	H_8	-	1.5	-
25° C	M_1	5.0	1.6	8.3
	M_8	-	0.3	1.7
	D_8	-	-	5.0
	H_8	-	0.2	-
37° C	M_1	5.3	1.3	9.3
	M_8	-	0.2	1.7
	D_8	-	-	7.2
	H_8	-	0.1	-

Table III-3. Packer X Cello Wrap Weiners at Retailers I, H, G and D. Total colony counts x 10^3 based on four day-old subsamples from Packer X. Four counts on Packer's subsamples are averaged and reported as the Mean (M_1). One subsample at each retailer was taken seven days later; reported as I_8, H_8, G_8 and D_8 respectively. The average of all retail counts is reported as M_8 for comparative purposes.

Incubation temperature	Total colony counts x 10^3 Sample site and age in days					
	M_1	M_8	I_8	H_8	G_8	D_8
10° C	1.0	73.9	100.0	180.0	12.5	3.3
25° C	3.1	556.0	1000.0	875.0	150.0	200.0
37° C	1.7	232.0	87.0	640.0	46.0	175.0

Table III-4. Comparative growth ratios based on data from Table III-3.

		Ratios				
10° C	Sample site and age	M_8	I_8	H_8	G_8	D_8
	M_1	73.9	100.0	180.0	12.5	3.3
	M_8	-	1.3	2.4	0.2	<0.1
	I_8	-	-	1.8	0.1	<0.1
	H_8	-	0.6	-	<0.1	<0.1
	G_8	-	0.8	1.4	-	<0.1
	D_8	-	30.3	54.6	3.8	-
25° C	M_1	179.3	322.6	282.0	48.3	64.5
	M_8	-	1.8	1.6	0.3	0.4
	I_8	-	-	0.9	0.1	0.2
	H_8	-	1.1	-	0.2	0.2
	G_8	-	6.6	5.8	-	1.3
	D_8	-	5.0	4.4	0.7	-

Table III-4 (cont.)

		M_8	I_8	H_8	G_8	D_8
37° C	M_1	136.4	51.2	376.5	27.1	102.9
	M_8	-	0.3	2.7	0.2	0.7
	I_8	-	-	7.3	0.5	2.0
	H_8	-	0.1	-	<0.1	0.3
	G_8	-	1.9	13.9	-	3.8
	D_8	-	0.5	3.6	0.3	-

Table III-5. Packer X Cryovac Wrap Weiners at Retailers D and F. Total colony counts x 10^3 four day-old subsamples from Packer X. The four counts on Packers subsamples are averaged and reported as the Mean (M_1) for initial count. One subsample at each retailer was taken eight days later; reported as D_9 and F_9 respectively. The average of both retail counts is reported as M_9 for comparative purposes.

	Total colony counts x 10^3			
Incubation temperature	Sample site and age of sample in days			
	M_1	M_9	D_9	F_9
10° C	17.2	7505.0	15000.0	11.0
25° C	717.2	12538.0	25000.0	76.0
37° C	990.0	12665.0	25000.0	330.0

Table III-6. Comparative growth ratios based on data from Table III-5.

		Ratios		
10° C	Sample Site and age	M_9	D_9	F_9
	M_1	435.0	869.5	0.6
	M_9	-	1.9	<0.1
	F_9	-	1363.6	-
	D_9	-	-	<0.1
25° C	M_1	17.5	20.9	0.1
	M_9	-	1.9	<0.1
	F_9	-	328.0	-
	D_9	-	-	<0.1
37° C	M_1	12.4	2.0	0.3
	M_9	-	76.0	0.3
	F_9	-	75.5	-
	D_9	-	-	<0.1

Guide to the Interpretation of Tables for Series IV and V

Use of bulked samples from packets of one code date at both packer and retailer allows presentation of the results of eight tests to be made in one table. For each code date the first site given is the packer (X or Y); below this is the site of retail storage (N, F, H, or G).

Results of previous series were based on the age since the code date was applied, i.e. since the wrap was placed on the meat. For purposes of comparison with the results of other series, the ratio tables are again based on the age of the packet. An additional column however is included in the tables of total counts, dividing age into "since cook" and "since wrap". This was made necessary by the realization that there is frequently a difference in the time interval between cooking and wrapping, which interval may be as long as ten days. Ratios based on the age of the cooked meat rather than of the packet might have been considerably higher.

Series IV

Table IV-1. Packer Y Cello Wrap Weiners at Retailer N, F, H, and G. Total colony counts x 10^3 based on quadruple sampling at both packer and retailer. All samples bore the same code-date. Four were left at one retailer for six days while the remaining four were tested the day of collection. The four subsamples were pooled for testing in each case.

Total colony counts x 10^3

Expt.	Site	Age (days) Since cooked	Since wrapped	Plate count agar incubated at 10° C	25° C	37° C	45° C
1.	Y	4	1	3.8	5.2	4.2	
	N	10	7	6.4	7.6	6.8	
2.	Y	1	0	1.7	7.8	6.7	
	N	7	6	0.8	4.3	2.5	
3.	Y	4	1	0.6	1.7	1.9	
	F	10	7	1.6	2.1	1.8	
4.	Y	4	1	2.5	1.4	1.2	0.1
	F	10	7	0.5	1.4	1.1	0.4
5.	Y	1	0	1.1	2.1	1.4	
	H	7	6	4.4	1.7	1.7	
6.	Y	1	0	1.6	2.9	4.2	
	H	7	6	1.1	0.8	1.2	
7.	Y	4	1	2.4	10.7	0.7	
	G	10	7	0.8	0.6	0.7	
8.	Y	4	0	0.6	3.5	3.7	
	G	10	6	1.2	3.2	2.7	

Table IV-2. Packer Y Cello Wrap Wieners. Range of total colony count x 10^3. Data from Table IV-1, based on 16 samples to each group.

Total colony counts x 10^3				
Group I	Days since wrapped			
	0 days		6 days	
Incubation temperature	Range	Average	Range	Average
10° C	0.6-1.7	1.3	0.8-4.4	2.6
25° C	2.1-7.8	4.1	0.8-4.0	2.6
37° C	1.4-6.7	4.0	1.2-2.7	2.0
Group II				
	1 day		7 days	
10° C	0.6-3.8	2.3	0.5-6.4	2.3
25° C	1.4-10.6	4.7	0.6-7.6	2.9
37° C	0.7-4.2	2.0	0.7-6.8	2.6

Table IV-3. Packer Y Cello Wrap Wieners. Comparative growth ratios from averages in Table IV-2 representing average population change on Y wieners at all four retailers during six storage days.

	Ratios* at		
	10° C	25° C	37° C
Group I	1.5	0.6	0.5
Group II	1.0	0.6	1.3
Average, retailers	1.3	0.6	0.9

*A ratio of less than unity represents a decrease in numbers; ratios greater than unity represent increases in over the six day storage period.

Table IV-4. Packer Y Cello Wrap Wieners. Comparative growth ratios for wieners at individual retailers. Data from Table IV-1.

		Ratios at		
Expt.	Retailer	10° C	25° C	37° C
1	N	1.7	1.4	1.6
2	N	0.5	0.5	0.4
3	F	2.4	1.3	0.9
4	F	0.2	0.0	0.9
5	H	3.8	0.8	1.2
6	H	0.7	0.3	0.3
7	G	0.3	0.1	1.0
8	G	2.8	0.9	0.7

Table IV-5. Packer X Cryovac Wrap Wieners at Retailer N, F, H and G. Total colony counts x 10^3 based on quadruple sampling at both packer and retailer. All samples bore the same code-date and were taken from the wrapping line together; four were left at one retailer for six days while the remaining four were tested the day of collection. The four subsamples were pooled for testing in each case.

		Age (days)		Total colony counts x 10^3 Plate count agar incubated at			
Expt.	Site	Since cooked	Since wrapped	10° C	25° C	37° C	45° C
9	X	1	0	10.0	211.0	189.0	
	N	7	6	170.0	300.0	600.0	
10	X	1	0	50.0	1200.0	4200.0	
	N	7	6	120.0	1860.0	2100.0	4300.0
11	X	1	0	2.2	2500.0	2500.0	
	F	7	6	5970.0	7180.0	7750.0	
12	X	1	0	2.5	500.0	500.0	5.0
	F	7	6	0.4	600.0	450.0	4.0
13	X	1	0	15.0	157.0	173.0	
	H	7	6	180.0	420.0	460.0	
14	X*	1	0	2.9	10.8	8.2	
	H*	7	6	20.0	65.4	62.6	
15	X	1	0	100.0	238.0	200.0	
	G	7	6	3.0	6.0	12.0	
16	X	1	0	3.0	166.0	121.0	
	G	7	6	8.5	36.0	64.0	

*These packets contained slim wieners (12 per lb) similar to those of packer Y. All other packets from packer X contained 9 per pound.

Table IV-6. Packer X Cryovac Wrap Wieners. Range of total colony counts x 10^3 on wieners of the same age from all four retailers. Each average based on 32 packets represents the count at one temperature. Data from Table IV-5.

	Total colony counts x 10^3			
	Days since wrapped			
	0		6	
Incubation temperature	Range	Average	Range	Average
10° C	2.0-1000.0	232.0	0.4-5970.0	809.0
25° C	10.0-2500.0	572.0	36.0-7180.0	1308.0
37° C	8.0-4200.0	986.0	62.0-7750.0	1439.0

Table IV-7. Packer X Cryovac Wrap Wieners. Comparative six day growth ratios based on average total counts from four retailers. Data from Table IV-6.

	Ratios at		
	10° C	25° C	37° C
Average, retailers	3.5	2.3	1.5

Table IV-8. Packer X Cryovac Wrap Wieners. Comparative six day growth ratios for wieners at individual retailers. Data from Table IV-5.

		Incubation temperature		
Expt.	Retailer	10° C	25° C	37° C
9	N	17.0	1.5	3.2
10	N	2.4	1.5	0.5
11	F	2722.0	2.9	3.5
12	F	0.2	1.2	0.9
13	H	12.0	2.6	2.7
14	H	6.9	6.1	7.6
15	G	<0.1	0.1	0.1
16	G	2.8	0.2	0.5

Table IV-9. Wieners from Packers X and Y. A comparison of wiener populations using ratio averages (individual retailers) from Table IV-8 and IV-4 respectively. Results are based on 128 samples, 64 from each packer. Of these, 32 samples from each packer were held at retail.

	Ratios X/Y at		
Retailer	10° C	25° C	37° C
N	8.9	1.5	1.9
F	1027.0	3.2	2.4
H	4.2	8.1	6.9
G	0.9	0.2	0.3

Table IV-10. Wieners from Packers X and Y. A comparison of wiener populations using ratios from Tables IV-7 and IV-3 respectively. Results are based on 128 packets, 64 from each packer.

	Ratios X/Y at		
Retailer	10° C	25° C	37° C
Average, retailers	2.8	3.7	1.6

Table IV-11. Counts* of Enterococci x 10^3 based on quadruple sampling of wieners at both packer and retailer. Counts were made on M-Enterococcus Agar (Difco).

Packer Y

Expt.	Site of Sampling	Age (days) since Cooked	Age (days) since Wrapped	Colony counts x 10^3 at 10° C	25° C	37° C	45° C
2	Y	1	0	-	-	0.4	-
	N	7	6	0.6	1.3	1.1	-
4	Y	4	1	0.1	0.1	0.1	0.1
	F	10	7	0.1	-	0.1	0.1
6	Y	1	0	-	-	0.1	-
	H	7	6	-	-	0.1	-
8	Y	4	0	-	-	-	-
	G	10	6	-	-	0.1	-

Packer X

10	X	1	0	-	-	4200.0	-
	N	7	6	200.0	1600.0	2960.0	-
12	X	1	0	-	-	1000.0	1.2
	F	7	6	-	-	1000.0	0.1
14	X**	1	0	-	-	14.0	-
	H**	7	6	-	-	63.4	-

Table IV-11 (cont.)

16	X	1	0	-	-	-	-
	G	7	6	-	-	0.1	-

*For parallel total counts see Tables IV-1 and IV-5.

**These packets contained 12 wieners/lb as did all Y packets. Packer X normally packed nine wieners per pound.

Series V

Table V-1. Cello Wrapped Sliced Cooked Ham from Packer Y at retailers N, F, H and G. Total colony counts x 10^3 based on quadruplicate sampling at both packer and retailer. Of eight subsamples collected from packer, four were left at one retailer for seven days. The remaining four were stored for one day at 3° C (U) then tested. In each case the four subsamples were pooled for testing. All subsamples came from wrapping line together and bore the same code date. Plate count agar was the medium used.

Total colony counts x 10^3

Expt.	Site	Age (days) Since cooked	Since wrapped	Plate count agar incubated at 10° C	25° C	37° C	45° C
1	Y*	7	2	271.0	22.4	17.5	
	N*	20	15	20000.0	7500.0	7500.0	2.0
2	Y	6	1	1.5	12.6	13.4	
	F	13	8	17.6	39.8	38.9	
3	Y**	2	0	0.3	6.6	7.9	
	H	9	7	1.0	11.0	9.0	
4	Y	7	1	1.0	31.4	28.8	
	G	14	8	5000.0	6000.0	2696.0	

*Delayed one week at retail.

**Tested immediately, not held 24 hours.

Table V-2. Packer Y Sliced Cooked Ham in Cello Wrap. Range of colony counts x 10^3 on ham packets of same age from all four retailers. Each average represents the count at one temperature on 16 packets. Data from Table V-1.

	Total colony counts x 10^3			
	one day since wrapped		ten days since wrapped	
Temp.	Range	Average	Range	Average
10° C	0.3-271.0	63.0	1.0-20000.0	6255.0
25° C	6.6-31.4	18.0	11.0-7500.0	3388.0
37° C	7.9-28.8	17.0	9.0-7500.0	2561.0

Table V-3. Packer Y Sliced Cooked Ham in Cello Wrap. Comparative seven day growth ratios based on average total counts from four retailers. Data from Table V-2.

	Ratios at		
	10° C	25° C	37° C
Average, retailers	99.3	188.2	150.6

*These ratios represent the increase in total population over a seven day period at retail. Thus at 25° C the average increase in population on ham for all four retailers was by a factor of 188.2.

Table V-4. Packer Y Sliced Cooked Ham in Cello Wrap Comparative seven day growth ratios for ham at individual retailers. Data from Table V-1.

		Ratios at		
Expt.	Retailer	10° C	25° C	37° C
1	N*	74.0	312.0	427.0
2	F	1.2	3.1	2.9
3	H	3.0	1.7	1.1
4	G	5000.0	194.0	93.0

*Time interval in this instance was 13 days.

Table V-5. Cello Wrapped Sliced Cooked Ham from Packer X at retailers N, F, H and G. Total colony counts x 10^3 based on quadruplicate sampling at both packer and retailer. Of eight subsamples collected from packer, four were left at one retailer for seven days. The remaining four were stored for one day at 3° C (U) then tested. In each case the four subsamples were pooled for testing. All subsamples came from the wrapping line together and bore the same code date. Plate count agar was the medium used.

		Age (days)		Total colony counts x 10^3 Plate count agar incubated at			
Expt.	Site	Since cooked	Since wrapped	10° C	25° C	37° C	45° C
5	X	5	1	64.0	13.1	7.2	
	N*	18	14	200000.0	125000.0	125000.0	3.0
6	X	5	1	1.2	1.5	1.1	
	F	13	8	2.0	200.0	190.0	
7	X**	5	0	0.3	0.8	0.7	
	H	22	7	138.0	119.0	80.0	
8	X	7	1	132.6	113.0	94.4	
	G	14	8	63000.0	250000.0	25000.0	

*delayed one week at retail.

**tested immediately, not held 24 hours.

Table V-6. Packer X Cello Wrapped Sliced Cooked Ham. Range of colony counts x 10^3 on ham packets of same age from all four retailers. Each average represents the counts at one temperature on 16 packets. Data from Table V-5.

	Total colony counts x 10^3			
	Average, 1 day since wrapped		Average, 10 days since wrapped	
Temperature	Range	Average	Range	Average
10° C	0.3-132.6	49.5	2.0-200000.0	6660.0
25° C	0.8-113.0	61.6	119.0-125000.0	93830.0
37° C	0.7-94.5	25.9	80.0-125000.0	93817.0

Table V-7. Cello Wrapped Sliced Cooked Ham from Packer X. Comparative seven day ratios based on average counts from four retailers. Data from Table V-6.

	Ratios at		
	10° C	25° C	37° C
Average, retailers	134.0	1523.0	3627.0

Table V-8. Cello Wrapped Sliced Cooked Ham from Packer X. Comparative seven day growth ratios for ham at individual retailers. Data from Table V-5.

	Ratios at		
Retailer	10° C	25° C	37° C
N*	30000.0	10000.0	17000.0
F	1.65	130.0	172.0
H	460.00	140.0	121.0
G	473.0	2214.0	264.0

*In this instance there was an interval of 13 days.

Table V-9. Cello Wrapped Sliced Cooked Ham from Packers X and Y. A comparison of ham populations from packers X and Y, using average ratios from Tables V-8 and V-4 respectively. Based on 64 packets, 32 from each packer of which 16 represent retailers.

	Ratios X/Y at		
Retailer	10° C	25° C	37° C
N	405.4	32.0	39.0
F	1.41	41.9	59.3
H	153.30	84.3	107.1
G	0.10	11.4	2.8

Table V-10. Cello Wrapped Sliced Cooked Ham from Packers X and Y. A comparison of ham populations from Packers X and Y using ratios from Tables V-7 and V-3 respectively. Based on 64 packets, 32 from each packer.

	Ratios X/Y at		
	10° C	25° C	37° C
Average, retailers	1.4	8.1	24.1

Table V-11. Counts* of Enterococci x 10^3 based on quadruple sampling of Cello Wrapped ham at both packer and retailer. Counts were made on M-Enterococcus Agar (Difco).

Packer Y

Expt.	Site of Sampling	Age (days) since Cooked	Age (days) since Wrapped	Colony counts x 10^3 at 10° C	25° C	37° C	45° C
1	Y	7	2	-	-	0.4	-
	N	20	15	0.1	-	7.6	0.1
4	Y	7	1	-	-	-	-
	G	14	8	-	-	12.0	-

Packer X

5	X	5	1	-	-	5.1	-
	N	18	14	0.1	10.0	28.0	0.1
8	X	7	1	-	-	-	-
	G	14	8	-	-	50.0	-

*For parallel total counts see Tables V-1 and V-5.

Table VI-1. Variation in initial colony counts on four packets of day-old wieners from Packer Y. All packets bore the same code date.

			Total colony counts x 10^3		
Group	Season	Wrap	10° C	25° C	37° C
1*	Winter	Cello	0.8	2.0	1.2
			0.9	2.0	1.6
			0.1	1.6	2.1
			0.5	1.1	1.7
2	Summer	Cello	34.5	60.0	125.0
			2.5	2.9	0.9
			2.3	4.6	3.2
			1.1	0.5	0.4
3	Summer	Cello	2.2	1.0	1.1
			2.5	1.4	1.3
			2.3	6.9	3.8
			1.7	0.9	1.0

*Counts from Group 1 were averaged to give initial count M, used in Table III-1.

Table VI-2. Variation in initial counts on four packets of day-old wieners from Packer X. All packets bore the same code date.

			Total colony counts x 10^3 at		
Group	Season	Wrap	10° C	25° C	37° C
1*	Winter	Cello	0.7	2.0	1.4
			0.4	2.6	2.1
			2.4	5.7	1.5
			0.9	2.4	2.0
2*	Summer	Cryovac	11.0	960.0	1300.0
			9.0	300.0	500.0
			48.0	1500.0	1500.0
			11.0	110.0	162.0
3	Summer	Cryovac	2.0	91.0	48.0
			0.1	170.0	108.0
			0.2	33.0	5.4
			0.4	160.0	119.0

*Counts from Groups 1 and 2 were averaged to yield the initial counts (M_1) in Tables III-3 and III-5 respectively.

Table VII. Variation in initial colony counts on four packets of cello wrapped ham from Packer X. All packets bore the same code date and were examined the day after wrapping.

	Total colony counts x 10^3 at incubation temps. of		
Group	10° C	25° C	37° C
1	21.0	16.7	1.1
	15.4	11.3	1.5
	45.0	41.0	0.9
	12.3	19.7	33.0
2	53.0	49.0	5.1
	770.0	58.0	4.2

Table VIII. Facilities available at the various retailers included in the surveys I, II and III.

Retailer	Facilities
A	Closed case at 36° F; walk-in at 35° F. Does own slicing as well as handling pre-sliced meats.
B	Open case at 27° F; walk-in at 33° F. No slicing facilities.
D	Open case at 20° F; walk-in at 34° F. No slicing facilities.
G	Open case at 31° F; walk-in at 34° F. No slicing facilities.
L	Closed case at 38° F; walk-in at 35° F. No slicing facilities.
M	Closed case. Store closed during course of investigation.
C	Open, double-deck case at 38° F overhead lights. Walk-in at 38° F. Does own slicing.
E	Open, double-deck case. Store closed during course of investigation.
F	Open counter at 40° F. Walk-in at 30° F.
H	Open counter at 29° F. Walk-in at 35° F.
I	Double-deck open counter at 37° F. Walk-in at 37° F. Delicatessen section with closed counter. Does own slicing.
J and K	Department stores, open counter type. Do own slicing.
U	University walk-in at 37.5° F.

Table IX. Facilities of retailers included in Series IV and V. An indication of age of stocks since wrapped is given.

Retailer	Facilities
N	Samples kept in walk-in at 38° F. Packets of the following types and ages were noted. Packer X wieners, ages 5, 8, 9, 11, 14 days. Packer Y wieners, ages 4, 5, 6, 9, 10, 12, 14 days. Packer X ham, none. Packer Y ham, ages 1, 5, 11, 13, 14, 17, 19, 32, 35 days.
F	Samples kept below counter at 34° F. Packets of the following types and ages were noted. Packer X wieners, ages 4, 4, 13, 14, 14, 16 days. Packer Y wieners, ages 4, 7, 8, 11, 14, 14 days. Packer X ham, ages 1, 6, 6, 7 days. Packer Y ham, ages 5, 6, 11, 12, 18 days.
H	Samples kept in walk-in at 35° F. Packets of the following types and ages were noted. Packer X wieners, ages 1, 5, 9, 11 days. Packer Y wieners, ages 5, 6, 6, 6, 7, 7 days. Packer X ham, none. Packer Y ham, ages 1, 6, 6, 6, 7, 7, 13, 14 days.
G	Samples kept in walk-in at 34° F. Packets of the following types and ages were noted. Packer X wieners, ages 2, 6, 12, 14 days. Packer Y wieners, ages 3, 4, 5, 5, 5 days. Packer X ham, none. Packer Y ham, 9, 14, 17 days.

BIBLIOGRAPHY

1. American Meat Institute Foundation (1960). Science of Meat and Meat Products.

2. Barnes, Ella M., Ingram, M. and Ingram, G. C. (1956). The distribution and significance of different species of faecal Streptococci in bacon factories. J. Appl. Bact. 19, 204-211.

3. Barnes, Ella M. (1956). Methods of Isolation of faecal Streptococci (Lancefield Group D) from bacon factories. J. Appl. Bact. 19, 193-203.

4. Biddle, G. R. (1961). Lamination with films. Dupont Technical Service Bull.

5. Brown, W. L., Vinton, C. A. and Gross, C. E. (1960). Heat resistance and growth characteristics of microorganisms isolated from semi-perishable canned hams. Food Res. 3, 345-350.

6. Buttiaux, R. and Moriamez, J. (1957). Bacteria indicative of faecal contamination. 2nd International Symposium on Food Micro. Cambs. 247.

7. Cameron, A. J. (1961). Vacuum and gas packaging in films. Dupont Technical Service Bull.

8. Clarenburg, A. (1955). Factors determining survival and development of Salmonella and other Enterobacteriaceae in semi-preserved meats. Ann. Inst. Pasteur. Lille. 7, 125.

9. Consumers' Union (1958). Anatomy of the hot dog. Consumer Reports, Sept., 467.

10. Deibel, R. H. and Niven, C. F., Jr. (1957). Studies on the microflora of commercial ham curing brines and their significance in curing. 2nd International Symposium on Food Micro. Cambs.

11. Deibel, R. H. and Niven, C. F., Jr. (1958). A motile lactobacillus in ham curing brines. Appl. Micro. 6, 323-327.

12. Deibel, R. H. and Niven, C. F., Jr. (1955). Reciprocal replacement of oleic acid and carbon dioxide in the nutrition of the minute streptococci and L. leishmannii. J. Bact. 70, 134-140.

13. Deibel, R. H. and Niven, C. F., Jr. (1955). Tween 80 and the minute streptococci. J. Bact. 70, 141-146.

14. Dolman, C. E. (1959). Epidemiology of Meat Borne Diseases. W. H. O. Mono 33.

15. Drake, S. D., Evans, J. B. and Niven, C. F., Jr. (1958). Microbial flora of packaged frankfurters and their radiation resistance. Food Res. 23, 291.

16. Drake, S. D., Evans, J. B. and Niven, C. F. Jr. (1958). Resistance of the Enterococci to heat and radiation. Bact. Proc. 24.

17. Drake, S. D., Evans, J. B. and Niven, C. F. Jr. (1960). The effect of heat and irradiation on the microflora of canned hams. Bact. Proc. 117.

18. Eisenhart, C. and Wilson, P. W. (1943). Statistical

methods and control in bacteriology. Bact. Revs. 7, 57.

19. Evans, J. B., Buettner, L. G. and Niven, C. F. Jr. (1957). Bacterial food poisoning. American Meat Institute Foundation. Circ. 29.

20. Food Engineering Editors (1955). Modern Meat Packing Practices. 2nd ed. McGraw Hill, New York.

21. Goldenburg, N., Sheppey, C. G., and Robson, J. N. (1955). Some tentative bacterial standards for canned hams. Ann. Inst. Pasteur. Lille., 7, 240.

22. Hayhurst, C. J. (1952). Research on flexible films for food packaging. Food Tech. 6, 454.

23. Heller, C. L. (1952). Standards for manufactured meats. J. Royal San. Inst. 72, Symposium.

24. Hobbs, Betty C. (1955). Laboratory investigation of non-sterile canned ham. Ann. Inst. Pasteur. Lille., 7, 190.

25. Ingram, M. and Barnes, Ella M. (1955). Streptococci in

pasteurized canned hams. Ann. Inst. Pasteur. Lille., 7, 101-114.

26. Ingram, M. (1955). Microbial association of semi-processed meats. Ann. Inst. Pasteur. Lille., 7, 32.

27. Ingram, M. (1955). Heat resistance of a micrococcus. Ann. Inst. Pasteur. Lille., 7, 146.

28. Ingram, M., Cheftel, M. H., Clarenburg, A., Lerche, D. R., Buttiaux, R. and Mossel, D. A. A. (1955). Suggestions regarding bacterial norms for non-sterile hams preserved in cans. Ann. Inst. Pasteur. Lille., 7, 252.

29. Ingram, M. (1957). Microbiology of meat curing brines. 2 nd International Symposium on Food Micro. Cambs. 121.

30. Ingram, M., Kitchell, A. G. and Ingram, G. C. (1957). Halophilic bacteria of bacon brines. 2nd International Symposium on Food Micro. Cambs.

31. James, N. and Sutherland, Marjorie (1941). A place for the plate count method as applied to soil. Iowa State Coll. Sci., 15, 375-383.

32. Jensen, L. B. (1944). Microbiological problems in the preservation of meats. Bact. Revs. 8, 161-188.

33. Jensen, L. B. (1949). Meat and Meat Foods. Ronald Co. New York.

34. Jensen, L. B. (1954). Microbiology of Meats. Garrard Co., Champaign, Ill.

35. Jepsen, A. (1959). Application of bacteriological and biochemical tests in the hygieneic judgement of meat and meat products. W. H. O. Mono. 33, 235 et seq., 420 et seq.

36. Jones, Audrey and Richards, T. (1952). Lipolysis by bacteria. II. Night blue and Victoria blue as indicators in lipolysis. Proc. Soc. Appl. Bact. 15, 82.

37. Kay, H. D. (1952). Bacterial standards for perishable foods. J. Royal San. Inst. 72, 388.

38. Kenner, B. A., Clark, H. F. and Kabler, P. W. (1961). Streptococci as a measure of faecal pollution . Appl. Micro. 9, 15.

39. Koch, S. O. (1959). Hygienic control of meat in markets and in food serving establishments. W. H. O. Mono. 33, 283.

40. Kraft, A. A., Ayres, J. C. (1952). Post-mortem changes in stored meats. III. Effect of packaging materials on keeping quality of self-service meats. Food Tech. 6, 8.

41. Maillet, M. (1955). Introduction to Symposium on semi-preserved meats, a definition. Ann. Inst. Pasteur. Lille, 7, 9.

42. Martin, J. F. (1950). The use of Rose Bengal streptomycin agar. Soils Sci. 60:215-232.

43. Mossel, D. A. A., Van Diepen, H. M. J. and DeBruin, A. S. (1957). Faecal Streptococci in foods. J. Appl. Bact. 20, 265.

44. Mossel, D. A. A. (1955). Importance of bacterial condition of ingredients used as minor components in some canned meat products. Ann. Inst. Pasteur. Lille., 7, 171.

45. Nevot, A. and Pantaleon, J. (1953). Bacteriological control of fresh meats. The paraffin block technique. 6th International Congress Micro. 7, Sezione 21, 300.

46. Niven, C. F. Jr. (1954). Bacteria in meat processing. American Meat Inst. Foundation. Circ. 12.

47. Niven, C. F. Jr. (1957). Sanitation problems in the meat industry. American Meat Inst. Foundation. Circ. 39.

48. Niven, C. F. Jr. (1955). Significance of Streptococci in canned hams. Ann. Inst. Pasteur Lille. 7, 120.

49. Ogilvy, W. S. and Ayres, J. C. (1951). Post-mortem changes in stored meats. II. The effect of atmospheres containing carbon dioxide in prolonging the storage life of frankfurters. Food Tech. 5, 300.

50. Ogilvy, W. S. and Ayres, J. C. (1953). Post-mortem changes in stored meats. V. The effect of carbon dioxide on microbial growth in stored frankfurters and characteristics of some microorganisms isolated from them. Food Res. 18, 121-130.

51. Pantaleon, J. and Rosset, R. (1955). Control of semi-preserved meats in the Department de la Seine. Ann. Inst. Pasteur. Lille., 7, 221.

52. Parry-Pritchard, D. E. (1952). Prevention of food poisoning. J. Royal San. Inst. 72, 1.

53. Paton, A. M. and Gibson, T. (1952). The use of hydrogenated fats in lipolysis media. Proc. Soc. Appl. Bact. iii.

54. Riemann, H. (1957). Survival of some Group D

streptococci in ham curing brines. 2nd International Symposium on Food Micro. Cambs.

55. Ross, A. D. and Thatcher, F. S. (1958). Bacteriological control of marketed precooked frozen foods in relation to public health. Food Tech. 12, 369-37.

56. Shewan, T. M. and Liston, T. A. (1955). A review of food poisoning caused by fish and fishery products. J. Appl. Bact. 18, 522.

57. Slanetz, L. W., Bartley, C. H. and Ray, V. A. (1956). Further studies on molecular filter procedures for determining numbers of Enterococci in water and sewage. Bact. Proc. 104.

58. Tefft, H. D. (1953). Sausage and Ready to Serve Meats. American Meat Inst. Univ. Chicago.

59. Thatcher, F. S. (1958). Microbiological standards for foods. Food Tech. 12, 117-122.

60. Thomas, S. and Grainger, T. H. (1952). Bacteria. Blakiston Co. New York.

61. Turner, Rosemary J. (1961). A Bacterial Survey of Certain Processed Meats. Canada Department of Health and Welfare, Project #606-7-34.

62. Wilson, G. D. (1961). Recent advances in meat research. Canadian Food Indust. Feb. 47.

63. Zaborowski, H., Huber, D. A. and Rayman, M. M. (1956). Evaluation of microbiological methods used for analysis of precooked frozen foods. Bact. Proc. 29.

APPENDIX

Conclusions regarding the significance of meat-borne Enterococci in public health and industrial practice.

From the foregoing summary of the experimental work it is evident that the Enterococci may constitute a problem not only to the meat packers but to public health officials. The sporadic appearance of very large numbers of faecal Streptococci from time to time tends to invalidate the use of random sampling as a control measure. It also complicates the setting up of bacterial standards based on a total count; high total counts may present an artifical picture unless these are paralleled by high counts on media selective for Enterococci. These organisms are difficult to eliminate in the course of processing. Furthermore they grow readily under cold storage conditions particularly when the meat is wrapped in an impermeable film.

Reference to the literature revealed that in recent years the Enterococci have been recognized as a major contaminant in processed meats although there appeared to

be few cases where wieners with very high counts of Enterococci have been reported (6, 25, 26, 54). This may be due to the fact that workers at packer laboratories may not observe them during routine surface counts. The colonies are minute and might tend to be overlooked particularly if low dilutions are used. As well, because of crowding and because of the masking effect of the diffuse meat inoculum the minute colonies are often difficult to recognize.

The presence of Enterococci as evidence of under-processing of ham has been of concern to a number of investigators (5, 16) and was noted frequently at the First International Symposium on Semi Preserved Meats at Lille in 1955 (25, 44). Even more concern may be occasioned by the very high counts of Enterococci in wieners. Wieners being smaller should require less heat treatment than a ham. Streptococcus faecium of bovine or porcine origin frequently survives smokehouse temperatures. This species appears as pink or white colonies on M-Enterococcus agar. In contrast S. faecalis of human origin, forms dark

red colonies on this medium. Streptococcus faecalis was seldom encountered in this survey, indicating relatively little human contamination. The prevention of access by S. faecium to meats can only be accomplished by the avoidance of agonal bacteraemia initially and by strict hygiene thereafter (25, 26, 54). Many workers believe that the Enterococci represent a surer index of faecal contamination in meats than do the coliforms which frequently fail to survive processing.

A process which fails to eliminate the microaerophilic faecal Streptococci must be regarded as inadequate because these organisms prepare the way for growth of the strictly anaerobic Clostridia with which they are often found in direct association in the gut (26). Ingram attributes one basic cause of meat spoilage to the failure to eliminate the Enterococci-Clostridial faecal association together with the micrococci (26). Not only are the Enterococci involved in the production of greening (25, 30) and of cheesy and sour flavours, but they are implicated in cases of food poisoning though there are relatively few outbreaks reported in view of their wide distribution.

Host susceptibility and the potency of various strains may vary. Enterococci may invade the bowel and may also produce an enterotoxin (60). Numbers are important, e.g. cases were caused by ham and beef bearing 3.5×10^6 and 4.5×10^6 Enterococci per gram respectively (140). Meat showing no obvious sign of spoilage and bearing 1×10^6 S. faecalis per gram was also responsible for an outbreak (51). The first case reported involved canned wieners.

The committee suggesting a code for ham at the 1955 symposium on semi-preserved meats (28) maintained that faecal Streptococci should be absent. They also gave as their reasons the following points: (1) Faecal origin, (ii) evidence of underprocessing, (iii) Clostridial association, (iv) implication in spoilage, (v) implication in poisoning.

The problem thus becomes one of how best to eliminate Enterococci from meat should they gain access to it. They tolerate a wide range of pH, a high salt soncentration (6) and a low Eh (54). Their resistance to penicillin enables them to increase as coliforms decline. Enterococci can survive freezing (55) and may grow at 0° C and 5° C (21, 25); they resist any practical level of irradiation (17) S. faecium

even more than S. faecalis. Heat appears to be the only effective agent though here again S. faecium is unusually resistant.

Goldenburg et al (21) found it necessary to maintain a temperature of 75° C for one hour in the centre of a ham to destroy an inoculum of 100 x 10^6 faecal Streptococci within the bone cavity; hams treated at 80° C were over-cooked and those held at 65° C still contained viable organisms.

Thatcher and Ross (55) reported that Enterococci survived in 10% of frozen pies cooked according to instructions on the packet. Meat particularly if it is fatty, protects bacteria from the effects of heat; a coccus isolated from ham withstood 40 mins at 150° F in vitro but when tested in meat it survived 400 mins at that temperature (5).

United States Federal Regulations call for an internal temperature of 66° C in the geometric centre of a ham but producers have voluntarily raised this to 71° C in an attempt to limit faecal Streptococci to 10/gm (46). Some workers believe one hour at 70° C to be too severe

and therefore impractical, it being better to rely on good hygienic practice than to manufacture dry friable ham (21). The same argument can be applied to wieners because pork is more susceptible to heat than most meats (41) particularly when it is comminuted, cured and smoked; protein breakdown releases fat from the emulsion and causes shrinkage. The internal temperature chosen must be a compromise between excessive shrinkage and the need for killing bacteria (26). If the packer cannot heat to kill he must attempt to eliminate the source of contamination (25).

Typical putrefaction is an anaerobic process and the addition of nitrate to meat is designed to maintain an aerobic condition in the bacteriological sense, preventing the growth of strict anaerobes and subsequent putrefaction (32). It would seem then that the use of ascorbate and of film wraps impermeable to air, such as Cryovac and Saran (20, p. 22) as a means of preventing oxidation in cured and smoked meats, is contradictory. Furthermore these packing films tend to retain surface moisture conducive to rapid microbial growth (47). Sixty percent moisture content is permitted in wieners and any lowering of water content would

result in a weight loss and consequent financial loss. Introduction of more permeable film wraps might also necessitate some modifications in government regulations governing weight loss.

Apart from the advent of refrigeration perhaps the greatest change in meat processing of late has been the trend to prepackaged convenience foods (40, 47, 62). It is questionable whether consumer demand for these should be paramount when such conveniences may induce health hazards. Questions of weight, color and attractive display must be weighed against possible dangers implicit in an anaerobic pack. Should the consumer prefer skinless wieners of sliced ham for convenience, wrapping in cello appears to be safer than wrapping in Cryovac. The use of the latter wrap may involve an unwarranted risk as it appears to favour the growth of Enterococci and would allow the growth of Cl. botulinum should it be present in the meat. Accordingly, unless a standard is set up at packing house level limiting the permitted number of Enterococci to less than ten per gram, it seems that the use of a breathing film wrap affords one of the few effective means of control in holding numbers to a safe level.