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The generation time, lag time, and minimum temperature of growth of coliform organisms on meat, and the implications for codes of practice in abattoirs

By M. G. SMITH

CSIRO Division of Food Research, Meat Research Laboratory, P.O. Box 12, *Cannon Hill, Queensland* 4170, *Australia*

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SUMMARY

The growth of coliform organisms on meat tissue from sheep carcasses processed in a commercial abattoir was investigated. The results indicated that for practical purposes the minimum temperature of growth of these organisms on meat may be taken as 8 °C. Equations were derived relating the generation time and the lag time of coliform organisms in raw blended mutton to the temperature at which the meat is held. Estimates of growth obtained with these equations were found to agree closely with the experimental results, especially at temperatures above 10 °C, and allowed the generation times and the lag times for all temperatures up to 40 °C to be calculated. These times were also found to agree closely with the times determined using a strain of *Escherichia coli* inoculated into blended mutton tissue. A strain of *Salmonella typhimurium* inoculated in the same way into blended mutton tissue gave longer generation and lag times at temperatures below 15 °C. Therefore, it is believed that the calculated tables of lag and generation times included in this paper can be used to determine the length of time raw chilled meat may be held afterwards at temperatures above the minimum temperature of growth without an increase in the number of any salmonella organisms present, and these times include a safety margin at each temperature.

The study indicates that the mandatory codes of practice presently applied in commercial abattoirs are too stringent. Maintaining the temperature of boning rooms at 10 °C or less does not appear to be necessary providing the meat is processed within the calculated time limits. A relaxation of the restrictions on boning room temperatures would decrease costs, increase worker comfort and safety and would not compromise the bacteriological safety of the meat produced.

INTRODUCTION

Statutory authorities responsible for public health have stipulated that animal carcasses must be refrigerated as soon as possible after slaughter and any cutting (fabrication) operations thereafter must be done in boning rooms maintained at 10 °C or less. These codes of practice were formulated to prevent any increase in the numbers of pathogenic bacteria such as salmonellas that might be present on the raw meat and are now mandatory in cattle and sheep abattoirs in many

countries, including Australia (Manual of Instruction for Meat Inspection and Meat Handling Procedures, 1976), Europe (E.E.C. Council Directives, 1975), and the United States of America (U.S.D.A. Meat and Poultry Inspection Manual, 1976).

After leaving the slaughter floor of an abattoir, animal carcasses are held at least overnight in chillers operating near zero, to reduce the internal temperatures sufficiently to comply with the present regulations. This often results in the surface fat becoming very hard and difficult to handle when boning out the meat next day. Understandably, the workmen involved would prefer the surface of the carcass to be warmed for a short period beforehand to soften the surface fat. However, this requires the carcasses to be held for a short period above 10 °C and therefore contravenes the present codes of practice imposed on abattoirs.

It is important to understand that the use of these codes of practice will not result in the removal of bacteria such as salmonellas from animal carcasses but aims to prevent those already present from multiplying. The question can be asked – are the codes of practice at present imposed on abattoirs realistic? If too severe they may only add to the cost of the meat produced and the inconvenience of the workmen without adding to the safety of the product.

The extent to which any micro-organism grows under specified conditions in a particular nutrient medium is a function of the temperature of the medium and the time for which that temperature is maintained (Thimann, 1955). Also, bacteria when initiating growth from a resting stage must pass through a lag phase before division takes place. Thus, even at temperatures at which organisms can grow there is a measurable initial time during which no cell division occurs. Therefore, even if animal carcasses after overnight chilling are then held for short periods at temperatures within the growth range of salmonella bacteria, there may still not be sufficient time for these organisms to increase in numbers.

Growth rates of *E. coli* in synthetic media have been determined previously (Ingraham, 1958; Monod, 1942) using optical density or turbidimetric measurements, but these may not correlate with the actual numbers of cells present or with the growth rates of these organisms on meat.

The experiments described here were undertaken to determine for practical purposes the minimum temperature of growth, the generation time, and the lag time of coliform organisms – and by analogy of salmonella bacteria – occurring on sheep carcasses processed in a commercial abattoir.

METHODS

Meat tissue

Meat samples (*c.* 5 g each) were taken from the neck tissue of about 50 sheep carcasses while still on the slaughter line in a commercial abattoir. Each piece of meat was rubbed over the anal area of the carcass from which it was cut. The samples were taken after the carcasses had passed the final washing and inspection stages.

Preparation of samples

Excess fat was removed from the tissue samples, which were then diced. The meat was placed in a blender jar (Sunbeam Corporation Ltd, Brisbane) and

blended in 50 g quantities at 15000 rev./min for 20 s. When all the meat had been blended it was thoroughly mixed and distributed into small polyvinyl chloride (PVC) pouches or 'sachets'. These were 8 cm by 15 cm with a film thickness of 35 μm. Approximately 5 g of blended meat was added to each sachet, which was then placed in a vacuum-packaging machine (M.P.I. Pty Ltd, Australia). To remove excess air from within the sachet a vacuum of about one kP was drawn and the sachet was heat sealed. The blended meat could then be easily distributed evenly throughout the sealed sachet to give a final thickness of meat of *c.* 1–2 mm. The result was a large surface area compared to the volume of meat present and was intended to resemble closely the surface of intact muscle. The initial count of coliform organisms in blended meat prepared in this way was usually *c.* 10^3 cells/g. Tests were always made on two sachets, and if the count was lower all the sachets were discarded. After preparation the sachets were spread on trays and placed in a chiller (0–2 °C) overnight to simulate the overnight chilling of sheep carcasses in an abattoir.

Inoculation with E. coli SF *and* Salmonella typhimurium *cells*

The meat tissue was obtained as before except that it was not rubbed over the anal area of the carcass. Tests showed that this resulted in initial counts of less than 10 coliform organisms/g in the blended meat. To inoculate the samples, cells of either a strain of *E. coli* or of *Salmonella typhimurium* from cultures incubated overnight in nutrient broth (Oxoid) at 37 °C were added before blending the meat tissue. Sufficient organisms were added to give *c.* 10^4 cells/g, a number greatly in excess of the natural contaminating coliforms. After preparation, these sachets were also chilled overnight (0–2 °C).

Incubation of samples

The sachets were incubated submerged in a water bath previously adjusted to the required temperature. The water was constantly stirred and the temperature maintained to an accuracy of $\pm 0{\cdot}2$ °C using Julaba Exotherm water-bath heaters. Tests using copper–constantan thermocouples (26 gauge) indicated that the meat in the sachets adjusted to the water temperature within 10 s of immersion.

Presence of oxygen in meat in sachets

PVC is readily permeable to oxygen, and tests on sachets in which the oxidation–reduction indicator methylene blue was added to the blended meat before sealing showed that the absence of oxygen was not a limiting factor in the growth of the organisms present, at least not until the later stages of logarithmic growth, and then only at temperatures above 30 °C. At this time the contents of the sachets were decolorized in parts, indicating anaerobic conditions, but this would not have affected the estimation of either the lag times or the generation times.

Determination of numbers of coliform organisms

Sachets of meat were removed from the water bath, dried, and three edges were cut away with sterile scissors. The meat tissue was transferred aseptically to a sterile blender jar, weighed, 45 ml of sterile 0·1 % peptone water was added (Oxoid

Neutralized Bacteriological Peptone, pH 7·0), and blended at 15000 rev./min for 20 s. This gave a 10^{-1} dilution. Then 0·1 ml aliquots of the blend, or of 10-fold dilutions of it, were pipetted on to the surface of MacConkey agar No. 3 plates (Oxoid), spread evenly with a sterile glass spreader, and incubated at 37 °C for 18–24 h. The agar plates were pre-dried by placing the open plate face down in a 37 °C incubator for 30 min. Red colonies at least 1 mm in diameter after 24 h incubation were counted as coliform organisms. This count was then adjusted by the actual weight of the meat added to the blender jar and the coliform count per gram of meat was calculated. In each experiment four sachets were tested initially to give an accurate count at zero time. Thereafter, sachets were tested in duplicate at the required intervals.

Determination of E. coli SF *cells*

These were estimated on MacConkey agar No. 3 incubated 18–24 h at 37 °C. A few colonies were picked and inoculated into Kohn's Two Tube Medium (Oxoid) to ensure they gave the reactions typical for *E. coli*. Experiments to determine the growth rate of this organism in nutrient broth at different temperatures showed that the counts on the MacConkey agar were usually about 0·3 log units lower than on a non-inhibitory medium (tryptone glucose yeast extract agar).

Determination of Salmonella typhimurium *cells*

Counts were made on Brilliant Green Agar (BGA) plates (Oxoid) containing sulphadiazine (May and Baker) 80 μg/ml. The pH of this medium was adjusted to 7·2, which made it less selective but also less inhibitory. Typical salmonella colonies were picked and inoculated into Kohn's Two Tube Medium (Oxoid) to ensure they gave the correct reactions. A few colonies were isolated from different experiments and submitted to the Salmonella Reference Centre, Adelaide, to confirm they were *S. typhimurium*.

pH

Determinations on the blended meat suspended in 0·1 % peptone water showed the pH before and after growth was always within the range 5·7–6·3. Thus the pH should not have had any detrimental effect on the rate of growth of the organisms present in the blended meat (Cohen & Clark, 1919).

Calculation of results

The $\log_{10}$ counts per g of meat were plotted on graphs against time (h), at each temperature tested. The lag time and the generation time, i.e. the time for the number of organisms to double during the logarithmic phase of growth, were calculated directly from each graph using the methods outlined by Meynell & Meynell (1965). The log time was calculated from the early growth phase on the graph where the plot of $\log_{10}$ number of cells against time showed a straight-line relationship. The only variable should have been the temperature of incubation, which thus determined the growth rate.

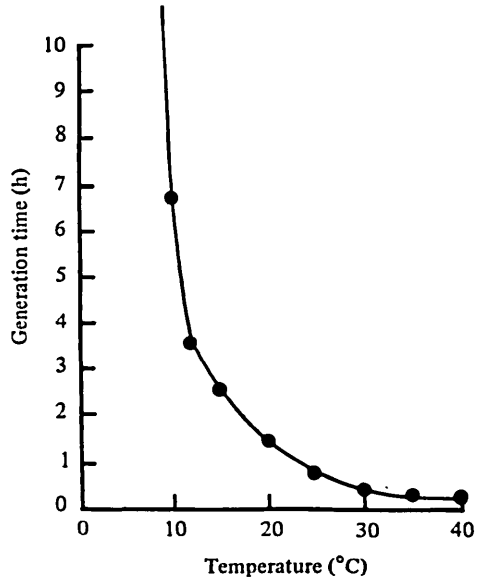

Fig. 1. Generation time of coliform organisms in sachets of raw, blended mutton tissue.

RESULTS

Generation time

The average generation times determined experimentally and the range of the results for each temperature investigated of coliform organisms on meat are given in Table 1. The average times were plotted against temperature (Fig. 1), and gave a hyperbolic curve which proceeded to infinity at a temperature below 10 °C.

The generation times of these organisms in meat at any temperature up to 40 °C can be estimated directly from this graph, but the values become increasingly imprecise at temperatures below 12 °C. Therefore the results were plotted according to the method of Ratkowsky *et al.* (1982), i.e. $\sqrt{R}$ against temperature, where R is generations/h. It was found the results up to and including 40 °C could be represented closely by a straight line. An equation to this line was derived by linear regression.

$$T = 3{\cdot}40 + 18{\cdot}58\sqrt{R} \quad (r^2 = 0{\cdot}98) \tag{1}$$

This equation was used to calculate the generation times of coliform organisms on meat at all temperatures up to 40 °C (Table 1).

Lag times

The average lag times determined experimentally and the range of the results for each temperature investigated of coliform organisms on meat are given in Table 2. The average times were plotted against temperature (Fig. 2), and gave a hyperbolic curve which proceeded to infinity at a temperature below 10 °C.

The lag times of these organisms in meat at different temperatures up to 40 °C can be estimated from this graph, but the values become increasingly imprecise at temperatures below 15 °C. Therefore the lag times obtained experimentally were plotted by the method of Ratkowsky *et al.* (1982), i.e. $\sqrt{1/L}$ against temperature,

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Table 1. *Generation time of coliform organisms in sachets of raw blended meat*

Temperature (°C)	Generation time (h)			
	Calculated from Equation (1)*	Experimental results (average)	No. of experiments	Range of experimental results
40	0·26	0·28	8	0·26–0·30
39	0·27			
38	0·29			
37	0·31			
36	0·32			
35	0·35	0·34	8	0·32–0·35
34	0·37			
33	0·39			
32	0·42			
31	0·45			
30	0·49	0·47	14	0·40–0·55
29	0·53			
28	0·57			
27	0·62			
26	0·68			
25	0·74	0·80	8	0·74–0·88
24	0·81			
23	0·90			
22	1·00			
21	1·11			
20	1·25	1·49	7	1·35–1·62
19	1·42			
18	1·62			
17	1·87			
16	2·17			
15	2·57	2·63	12	2·25–2·95
14	3·07			
13	3·75			
12	4·67			
11	5·98			
10	7·93	6·68	4	6·25–6·90
9	11·01			
8	16·31			
7	(26·64)			
6	(51·07)			
5	(134·85)			
4	(958·94)			
3	—			
2	—			
1	—			

* Equation (1): $T = 3{\cdot}40 + 18{\cdot}58\sqrt{R}$.

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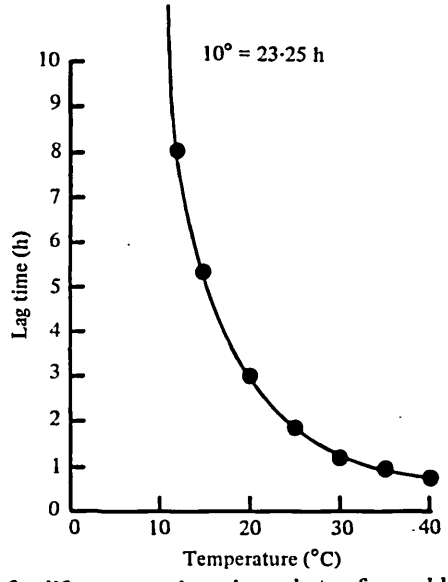

Fig. 2. Lag time of coliform organisms in sachets of raw, blended mutton.

and it was found that the results up to and including 40 °C could be represented closely by a straight line. An equation to this line was derived by linear regression.

$$T = 3{\cdot}00 + 29{\cdot}09\sqrt{1/L} \quad (r^2 = 0{\cdot}96) \tag{2}$$

This was used to calculate the lag times of coliform organisms on meat at all temperatures up to 40 °C (Table 2).

Comparison of results

The generation times (Table 3) and the lag times (Table 4) of coliform organisms in meat tissue found experimentally at a number of temperatures, and those calculated by the method of Ratkowsky *et al.* (1982) for these organisms, are compared with those found experimentally for a strain of *E. coli* and a strain of *S. typhimurium*.

DISCUSSION

In the present experiments the growth rate of coliform organisms naturally present on sheep carcasses processed in a commercial Australian abattoir were studied. Coliform bacteria are defined as those organisms which form red colonies at least 1 mm in diameter on the surface of MacConkey agar plates after 18–24 h incubation at 37 °C. A selective medium had to be used to detect the coliforms present as they were part of the unknown microflora normally present on the sheep carcasses. It is believed the growth characteristics of these organisms resemble those of mesophiles such as *E. coli* and salmonella strains.

Experiments using a pure culture of *E. coli* showed that the counts were usually about 0·3 log unit lower on the MacConkey agar than on a non-selective nutrient medium but, as the difference in the results was constant throughout the growth cycle, this would have made no difference to the generation times or the lag times

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Table 2. *Lag time of coliform organisms in sachets of raw blended meat*

Temperature (°C)	Lag time (h)			
	Calculated from Equation (2)*	Experimental results (average)	No. of experiments	Range of experimental results
40	0·62	0·74	8	0·60–0·90
39	0·65			
38	0·69			
37	0·73			
36	0·78			
35	0·83	0·96	8	0·70–1·60
34	0·88			
33	0·94			
32	1·01			
31	1·08			
30	1·16	1·19	9	1·00–1·20
29	1·25			
28	1·35			
27	1·47			
26	1·60			
25	1·75	1·92	5	1·70–2·00
24	1·92			
23	2·12			
22	2·34			
21	2·61			
20	2·93	3·00	7	2·50–3·50
19	3·31			
18	3·76			
17	4·32			
16	5·01			
15	5·88	5·90	11	4·50–7·00
14	6·99			
13	8·46			
12	10·45			
11	13·22			
10	17·27	23·25	4	20–29
9	23·51			
8	33·85			
7	(52·89)			
6	(94·03)			
5	(211·56)			
4	(846·23)			
3	—			
2	—			
1	—			

* Equation (2): $T = 3{\cdot}00 + 29{\cdot}09 \sqrt{\frac{1}{L}}$.

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Table 3. *Comparison of generation times of coliform organisms*, E. coli SF *and* S. typhimurium *cells, in blended meat at different temperatures*

	Generation time (h)			
		Experimental results		
Temperature (°C)	Calculated times*	Coliforms	*E. coli SF*	*Salmonella typhimurium*
40	0·26	0·28	0·30	0·30
35	0·35	0·34	0·37	0·39
30	0·49	0·47	0·52	0·49
25	0·74	0·80	0·78	0·75
20	1·25	1·49	1·40	1·60
15	2·57	2·63	2·60	2·65
10	7·93	6·68	6·90	9·65
8·2	14·98	17·80	17·20	—

— Organisms did not grow.
* From Equation (1) (Ratkowsky *et al.* 1982).

Table 4. *Comparison of lag times of coliform organisms*, E. coli SF *and* S. typhimurium *cells, in blended meat at different temperatures*

	Lag time (h)			
		Experimental results		
Temperature (°C)	Calculated times*	Coliforms	*E. coli SF*	*Salmonella typhimurium*
40	0·62	0·74	1·40	0·70
35	0·83	0·96	1·20	1·20
30	1·16	1·19	1·50	1·30
25	1·75	1·92	2·00	1·80
20	2·93	3·00	3·20	3·35
15	5·88	5·90	6·10	7·00
10	17·27	23·25	27·00	45·00
8·2	31·30	42·00	40·00	—

— Organisms did not grow.
* From Equation (2) (Ratkowsky *et al.* 1982).

determined by the method of Meynell & Meynell (1965). Similarly, the counts for a strain of *S. typhimurium* grown in pure culture were about 0·3 log unit lower on BGA plates (pH adjusted to 7·2) than on a non-selective medium, and again this difference was constant throughout the growth cycle.

Although salmonella bacteria are sometimes present on animal carcasses, the actual numbers of these cells are usually very low (Smith, unpublished information). Far more coliform organisms are usually present and, if it is accepted that these organisms grow at least as fast as salmonellas, then results obtained using coliforms may be used to reflect possible increases in salmonella organisms under similar conditions. Certainly the generation times of coliform organisms found in the present study (Table 1) compare closely with those found by Barber (1908) for what was then termed *Bacillus coli*, and by Shaw & Nicol (1969) for *E. coli* and

S. oranienberg on meat. Unfortunately, as far as the author is aware, no information has been published on the lag times of these organisms on meat over a range of temperatures.

It is emphasized that coliform organisms should not be regarded as a health hazard nor that their numbers will indicate the presence or absence of salmonellas. Nevertheless, if the growth characteristics are similar, then if conditions permit an increase in the numbers of coliform organisms, a similar increase may occur in the numbers of any salmonella bacteria that might be present.

The growth medium was lean sheep tissue taken from carcasses in a commercial abattoir after they had passed the final washing and inspection stages. Thus the organisms present should represent the normal microflora found on such carcasses. For each experiment, samples were taken from at least 50 individual animals and thoroughly mixed to overcome possible variation between animals. The experiments were designed so that all the factors affecting the growth of coliform organisms on the surface of meat tissue were kept constant except the temperature of incubation, and should closely simulate the surface of sheep carcasses after overnight chilling.

The average generation and lag times obtained experimentally were plotted against temperature (Figs. 1 and 2). The results were also treated mathematically by the method developed by Ratkowsky *et al.* (1982) in an attempt to obtain accurate estimates of the generation and lag times, especially at the lower temperatures.

The calculated generation and lag times compared with the experimental results are shown in Tables 1 and 2. There is good agreement in the results from 10 °C upwards, and at lower temperatures the generation and lag times are so prolonged as to be of no practical significance in the normal treatment of meat in an abattoir.

Shaw, Marr & Ingraham (1971) found that the minimum temperature of growth of a strain of *E. coli* in liquid nutrient medium lay between 7·5 and 7·8 °C, and Mackey *et al.* (1980) observed no growth of salmonella strains on beef at 7·5 °C. However, there have been reports in the literature that salmonellas can grow on laboratory media at temperatures substantially below 8 °C. A concise summary of these observations was given by Mackey *et al.* (1980). More recent work by Mossel, Jansma & de Waart (1981) has indicated that a number of salmonella strains can grow on buffered glucose tryptone soya peptone agar plates at 7 °C, and a few can grow on this medium at temperatures down to 4 °C. Interestingly, this agrees with what appears to be the minimum temperature of growth of coliforms on meat obtained in the present studies by the method of Ratkowsky *et al.* (1982) given by Equations 1 and 2 and tabulated in Tables 1 and 2. However, this may be only coincidence, and these authors do not claim that results obtained with their method should be extrapolated beyond observed values.

Even if some salmonella strains can grow on meat at temperatures below 8 °C the growth rate must be very slow. Therefore for practical purposes the minimum temperature of growth may be taken as 8 °C. In the present study the strain of *S. typhimurium* used was unable to grow in blended mutton tissue at 8·2 °C (Tables 3 and 4). Unless the meat was to be held for several days at or near 8 °C any increase in the numbers of coliforms, and hence of salmonellas, would be undetectable. However, the contaminating psychrotrophs, for example *Pseudomonas* spp., grow

much faster at these low temperatures, rapidly overgrowing any mesophiles present and quickly causing spoilage. A similar view was expressed by Mossel, Jansma & de Waart (1981).

Above the minimum temperature of growth, coliforms and salmonellas can initiate growth, and consideration must be given to the length of time meat can be held without an increase in the number of these bacteria. From Tables 3 and 4 it can be seen that if the calculated generation times (Equation 1) and the calculated lag times (Equation 2) are added, the result is always less than that found experimentally for the natural coliform organisms, the *E. coli SF* or the *S. typhimurium* cells in blended mutton. Thus, after overnight chilling it should be possible to hold meat for these times at these temperatures before one cell division could occur, and this includes at least a small safety margin at each temperature. Therefore the following holding times would be allowable for chilled meat before there could be any increase in the numbers of coliforms, and hence of salmonellas – at 10 °C about 25 h; at 15 °C about 8·4 h; and at 20 °C about 4·1 h. In the same way, other time/temperature relationships can be easily established from Tables 1 and 2.

The results calculated from Equations (1) and (2) shown in Tables 1 and 2 give an indication of the growth of coliform organisms on raw meat held for any length of time at any temperature up to 40 °C. Of course, if the temperature of the meat is not constant, then total growth would be represented by an integration of growth over a series of temperature increments. If the expected growth, including the time for the organisms to overcome the lag phase, indicates that not even one division of coliform cells could have occurred, then the processing conditions may be considered satisfactory.

Under no circumstances should salmonellas be allowed to proliferate on meat produced for human consumption. However, neither is it necessary for conditions to be overly stringent. For example, if animal carcasses after overnight chilling are processed promptly and the meat is not allowed to accumulate in the boning or cutting rooms, it should be possible to maintain these rooms at temperatures above the present mandatory 10 °C. This would certainly reduce refrigeration costs and increase worker comfort and safety without being detrimental in any way to the safety of the meat produced. However, it would be necessary to clean the working surfaces in the room at intervals throughout the working day to prevent growth of both pathogenic and spoilage bacteria on these areas.

This becomes especially important in the context of 'hot-boning', in which the meat is removed from animal carcasses coming directly off the slaughter floor or with only a short period of chilling. Indeed, it would then be quite illogical to maintain boning rooms at a temperature of 10 °C, thus chilling the walls, floors, ceilings, rails, tables and workers, etc., while the actual temperature of the meat is substantially higher. Of course, the meat would still have to be chilled quickly enough to prevent the growth of salmonellas, but the emphasis should be on chilling the meat to accomplish this, and not on chilling the surroundings.

Although all the results reported here were obtained using mutton tissue, experiments carried out using beef tissue gave similar results. Therefore it appears that the growth rate of coliform organisms on mutton and beef is comparable and the same calculations can be used. Pathogenic bacteria other than salmonellas may

sometimes contaminate animal carcasses. As far as the author is aware, none of these has been shown to grow faster than salmonellas or coliforms aerobically. Therefore, providing these latter organisms cannot multiply, other pathogens should also be kept in check.

All of the present experiments were made using blended meat enclosed in small sachets of polyvinyl chloride. It is still important to determine how accurately the results reflect the situation on carcasses, but it is very unlikely that any faster rate of growth would be obtained. Also, no account has been taken of the effect of drying of the surface tissues of cattle or sheep carcasses under normal conditions in an abattoir, but again this could only lead to slower growth of the organisms present (Scott, 1936; Scott & Vickery, 1939).

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