Total and Intramuscular Bacterial Populations of Carcasses and Cuts

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In recent years there has been relatively little attention given to the question of the extent to which bacteria occur within muscle tissues. Although the available evidence has been far from conclusive, the ubiquitous presence of an intramuscular bacterial population which includes pathogenic species has often been assumed. The following passage by Ingram & Dainty (1971) gives an account of this traditional view:

> "There are important differences between spoilage under warm and under chill conditions. Under warm conditions suitable for the growth of mesophiles, the predominant spoilage species are clostridia, which grow within the meat so rapidly as to precede spoilage at the surface. Under cool conditions this is not the case, because there are few psychrotrophic clostridia and such species as are cold-tolerant grow relatively very slowly under these conditions. Spoilage of chilled meat is therefore usually caused by the development of more or less aerobic species predominantly on the surface. Broadly speaking, these groups of bacteria have differenct origins: the clostridia and other faecal bacteria come mainly from the gut of the animal, maybe by an internal route, while the aerobes and facultative anaerobes come from external contamination like soil and are often psychrotrophic. It is useful to distinguish these origins as 'intrinsic' and 'extrinsic,' respectively."

This description of two microbial populations in meat is more precise than is usual, so it is unfortunate that most of the comments on intrinsic bacteria are misleading. It is notable that there is no consideration of any relationships between intrinsic and extrinsic floras, nor is there any indication of what is meant by an internal route. However, it is only by examination of these points that it is possible to develop a better understanding of the real extent and significance of bacterial contamination of deep tissues.

Aerobic, Extrinsic Floras

Most meat is stored for some period in air at chill temperatures, so there is a large literature on the floras that develop under these conditions. There is very little data on aerobic floras of meat at higher temperatures. At chill temperatures the spoilage flora is remarkably homogeneous invariably being composed of Gram-negative rods with species of *Pseudomonas* predominating, although they rarely form a large part of the initial flora. They remain the dominant species on high ultimate pH, dark, firm, dry (DFD) meat, and with holding temperatures up to at least 20°C. Even at 30°C they are still a major part of the spoilage flora (Table 1). The reason for the dominance by pseudomonads of aerobic meat spoilage floras seems to be that they grow faster than their competitors over a wide range of temperature and pH (Gill & Newton, 1977).

Table 1. Percent Compositions of Final Aerobic Spoilage Floras from Beef Held at Different Temperatures

	Storage Temperature °C		
	10	*20*	*30*
Pseudomonas	89	60	19
Acinetobacter	5	11	44
Moraxella	–	10	1
Micrococcus	–	8	–
Enterobacteriaceae	4	5	36
B. thermosphacta	2	5	–
Others	–	1	–
Mesophils	2	36	58

Most species of bacteria growing on meat will preferentially utilize the small quantity of glucose present. When this is exhausted, amino acids are attacked and their breakdown products are detected as spoilage odours (Gill, 1976). Pseudomonads grow equally rapidly when metabolizing glucose or amino acids. Their growth is also unaffected by changes of pH in the range found in meat. This means that variation in meat composition has no effect upon pseudomonad growth and explains why the flora of DFD meat, which can be devoid of glucose and has a high pH, is not greatly different from that which occurs on normal meat. The pseudomonads tend to have an advantage in growth rate over competing species at all temperatures up to 20°C (Fig. 1). It is only at higher temperatures that mesophils and many psychrotrophs can both initiate growth and grow sufficiently rapidly to compete successfully with the small numbers of pseudomonads present in the initial flora.

The aerobic spoilage flora of fresh meat is therefore remarkably stable to changes in the meat composition and the

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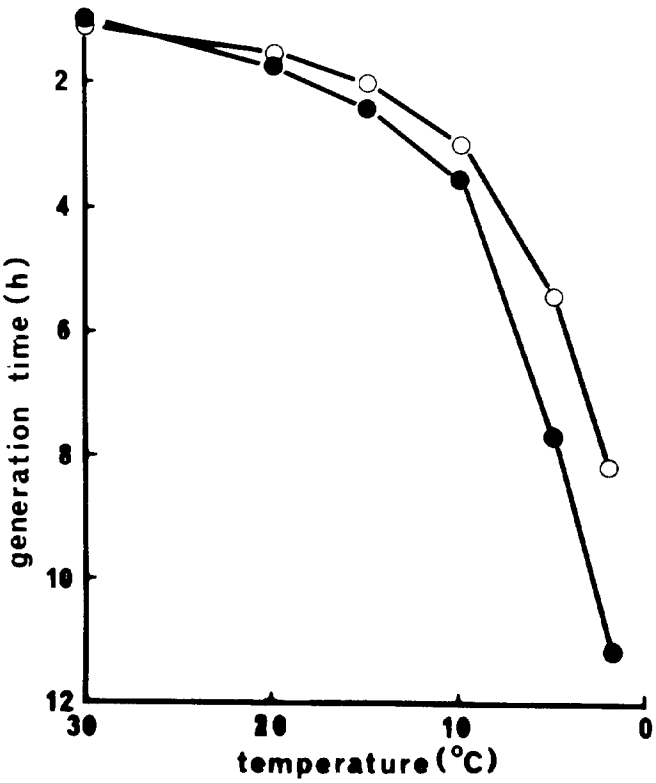

Figure 1. Effect of temperature on rate of aerobic growth of *P. fluorescens* (○) and Enterobacter (●).

environment, so it is possible to make reasonable predictions regarding the behaviour of other species within such a flora on the basis of their individual growth rate response to variations of pH, temperature and substrates.

Anaerobic, Extrinsic Floras

With the increasing use of vacuum packaging, many more cuts are being held in an anaerobic environment. Unlike the situation with aerobic storage, the floras formed under anaerobic conditions are subject to major changes with temperature and important modifications as a result of elevated pH.

Bateria growing aerobically have substrates for oxidation available in excess. Aerobic growth ceases because of oxygen limitation while most substrates are still abundant. The situation is very different under anaerobic conditions, where most organisms can ferment only a few of the substances present in meat, and anaerobic growth at chill temperatures ceases because of substrate exhaustion at the meat surface (Newton & Gill, 1978).

Changes in the storage temperature produce drastic alterations of the anaerobic spoilage flora. With temperatures up to about 15°C the flora is dominated by psychrotrophic lactobacilli, which not only outgrow competitors but inhibit them by production of an antimicrobial agent. At 20°C the lactobacilli are displaced by enterobacteriaceae, both mesophilic and psychrotrophic strains being present, while at 30°C these are in turn displaced by clostridia and mesophilic lactobacilli (Table 2).

Table 2. Percent Composition of Final Anaerobic Floras from Beef Held at Different Temperatures

	Storage Temperature °C		
	10	*20*	*30*
Lactobacillus	92	20	33
Enterobacteriaceae	1	80	5
B. thermosphacta	7	–	–
Clostridium	–	–	62
Mesophils	0	50	99

Increase in pH can also have important effects on the composition of the anaerobic spoilage flora by allowing growth of species which are partially or totally inhibited at the normal ultimate pH of meat. These include *Yersinia enterocolitica*, some strains of which can be pathogenic; *Alteromonas putrefaciens*, an organism which produces large quantities of H_2S under anaerobic conditions and so causes early spoilage by "greening"; and *Enterobacter liquifaciens*, a potent producer of spoilage odours (Gill & Newton, 1979). The composition of the spoilage flora in vacuum packages can be further affected if the packaging film permits oxygen to enter at a rate sufficient to support aerobic growth of organisms like *Bronchothrix thermosphacta* or pseudomonads (Newton & Rigg, 1979).

The effects on the microbial flora of variations in the microbial environment are very much greater in vacuum-packaged meat than in meat stored in air. Prediction of the behaviour of any species on vacuum-packaged meat would therefore be more difficult because of the number of factors that have to be considered.

Interactions Between Extrinsic and Intrinsic Floras

There are two ways in which extrinsic bacteria could influence any intrinsic flora. Extrinsic bacteria could penetrate into the meat and so contribute to an intrinsic flora, or, if extrinsic bacteria are confined to the meat surface, their activity could alter the environment within the muscle tissue.

By embedding blocks of meat in agar to give a bacteria-tight seal around the edge of each block, it was possible to inoculate one surface and determine the conditions necessary for bacteria to penetrate through the meat and emerge at the other surface (Gill & Penney, 1977). Penetration was achieved by any bacterial species which produced proteolytic enzymes. In sections through penetrated samples the bacteria were found between muscle fibres and their surrounding coat of connective tissue, neither of which showed any sign of disruption (Plate 1). The area traversed by the bacteria contains an amorphous ground substance which is difficult to demonstrate by staining. It is this material which seems to be degraded by proteolytic enzymes before bacteria can move through the tissue.

Since many of the bacteria found on meat are proteolytic, this might suggest that invasion from the surface could be a real problem. However, production of extracellular proteases by bacteria only occurs under certain conditions and usually

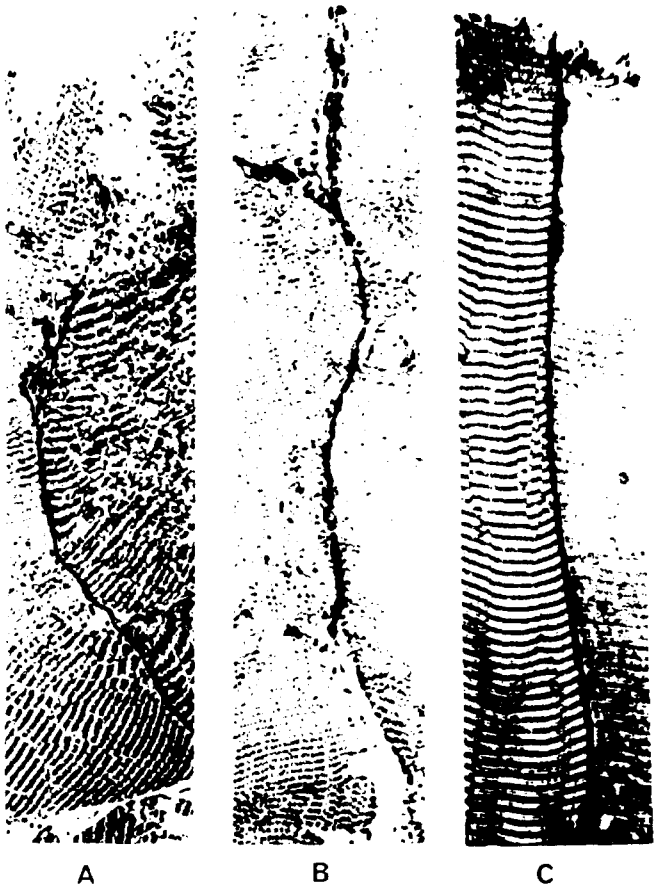

Plate 1. Section vertical to the inoculated surface of meat inoculated on one surface with *P. fluorescens*. (A) proteolytic stain, inoculated surface; (B) proteolytic stain, centre of meat sample; (C) non-proteolytic stain, inoculated surface.

only during the late logarithmic phase of growth (Glenn, 1976). This means that bacteria of the aerobic flora do not produce proteolytic enzymes until spoilage is at an advanced stage. With anaerobic flora, the lactobacilli are non-proteolytic, so at chill temperatures no penetration is likely to occur even in packages held for six to eight weeks after maximum bacterial densities are reached. At higher temperatures proteolytic organisms can predominate, so penetration could occur. This situation has not been investigated, but usually proteolysis is accompanied by amino acid utilization and hence by production of spoilage odours, so it seems unlikely that vacuum-packed meat could be penetrated by bacteria without clear indications of spoilage. For all practical purposes, therefore, the extrinsic flora is confined within 1 or 2 mm of the meat surface.

The effect on the deep tissues of bacterial activity at the meat surface is also unlikely to be of any significance until spoilage is well advanced. The muscle tissue is anaerobic except for a few millimeters just below the surface. Bacteria would ultimately prevent access of oxygen to the outer layer of muscle, but this would only occur with bacterial cell densities near to spoilage levels. Similarly, there is little effect of bacteria on the deep meat composition until spoilage numbers are reached. The only substance whose concentration significantly alters before spoilage is glucose, and again it is only near the surface that any large changes in glucose concentration will occur (Fig. 2).

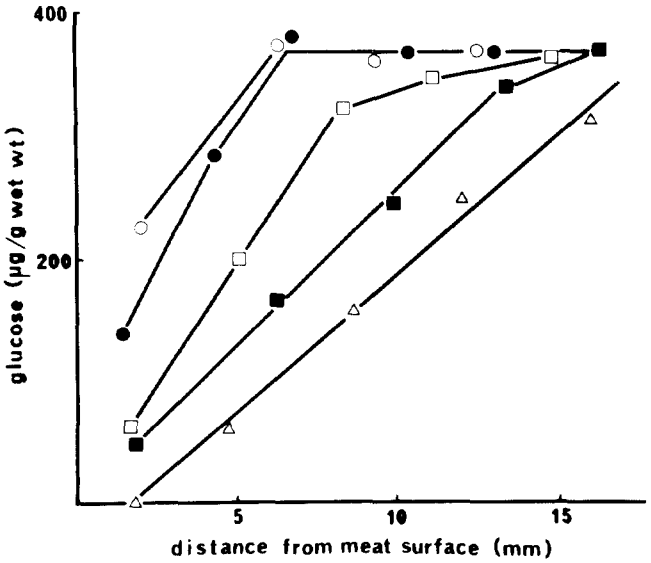

Figure 2. Concentration gradients of glucose in meat with *P. fluorescens* on the surface at densities of 2.7×10^7 (○); 6.3×10^7 (●); 3.2×10^8 (□); 1.1×10^9 (■); and 2.8×10^9 (△).

Intrinsic Bacteria

The extrinsic flora does not contribute to, nor is it likely to influence, any intrinsic flora, which can therefore be considered as a completely separate entity. In discussion of intrinsic floras the first points that need to be resolved are the incidence and compositions of intrinsic floras.

Bacteriologists originally thought that the tissues of healthy animals would be sterile at the time of slaughter, but this assumption was undermined at the beginning of the present century by a number of workers who claimed to have demonstrated the presence of bacteria in large percentages of carcasses. Textbooks tend to give the impression that there is ample evidence for the widespread occurrence in meat of an intrinsic bacterial population composed of numerous species. In fact, the published data are far from unambiguous. The available data on cattle are shown in Table 3. Similar data exist for sheep, pigs and a number of species of laboratory animal (see Gill, 1979). This evidence could equally well be used to support any one of three mutually exclusive views of deep tissue contamination. These are:

1. that most tissues from a majority of carcasses contain a comparatively large ($>10^2$/g) mixed population of intrinsic bacteria;
2. that some tissues, notably liver, spleen and lymph nodes, from a minority of carcasses contain a small number (1/g) of clostridia;
3. that deep tissue is generally sterile.

Table 3. Reports on the Bacteriological Condition of Deep Tissues of Cattle

No. of animals	*Tissue examined*	*Positive animals %*	*Bacteria isolated*	*Bacteria/g of tissue*	*Reference*
23	Muscle Lymph nodes	7 60	Gram + & − organisms	10^2-10^4	1
11	Muscle	27	Gram + rods & cocci	10^3	2
15	Liver	"many"	cocci & clostridia	N.D.	3
100	Muscle Lymph nodes Liver	61 58 32	clostridia	10	4
29	Muscle Lymph nodes Liver	5 10 15	clostridia	1	5
100	Liver	12	clostridia	N.D.	6
50	Liver Spleen	4	N.D.	N.D.	7
N.D.	Lymph nodes	3	clostridia	10	8
6	Muscle	0	sterile	0	9
6+	Muscle	0	sterile	0	10
N.D.	Muscle	0	sterile	0	11

1) Lepovetsky *et al.* 1953; 2) Vanderzant & Nickelson, 1969; 3) Smith & Jasmin, 1956; 4) Narayan, 1966; 5) Zaghaevskii, 1973; 6) Canada & Strong, 1964; 7) Von Stuker *et al.*, 1977; 8) Moran *et al.*, 1973; 9) Buckely *et al.*, 1976; 10) Ockerman *et al.*, 1969; 11) Radouco-Thomas *et al.*, 1959.

It has been suggested that these contradictory findings arise because of major differences between species. However, for all animals that have been studied by more than one group there is evidence for both the presence of bacteria and for sterile tissue. To resolve these contradictions consideration must be given to the mechanisms by which bacteria could contaminate the deep tissues of carcasses from healthy animals.

Contamination could conceivably occur before, during and/or after death of the animal. These possibilities are most conveniently considered in the reverse order to events.

Post Mortem Contamination

Since deep tissue invasion by the extrinsic flora cannot occur until spoilage is well advanced, the only post mortem mechanism of interest is invasion by bacteria from the intestine. The idea of post mortem invasion of other tissues by bacteria from the intestine originated with human pathology as an attempt to explain discrepancies between clinical symptoms and findings at autopsy. How, in the absence of circulating blood, bacteria were supposed to move rapidly from the intestine to other organs and the musculature was never explained, the case resting solely on the culture of bacteria from tissues where they had no business being. Some recent analyses of pathological data have rejected the concept of rapid post mortem invasion from the intestine, but the idea has continued to be used in discussions on meat hygiene.

We carried out the obvious, though previously unperformed, experiment of holding uneviscerated carcasses at room temperature for extended periods, then examining the deep tissues for the presence of bacteria. It seemed essential with such experiments that deep tissue samples were removed aseptically and that very small numbers of bacteria could be detected. The first requirement could be met by removing meat samples to a sterile blending cup direct from the carcass after the surface of the meat had been seared to a depth of 3 to 4 mm with a hot iron. Merely flaming the meat surface does not guarantee destruction of bacteria on the surface, and any manipulation of the material to be sampled, such as removing a portion of the carcass to the laboratory before sampling, increases the chance of extraneous contamination. To detect small numbers of contaminating bacteria, the homogenized samples were incubated in the blender cups at 37°C for 24 h. Under these conditions even a single bacterium in the 20 g sample would give rise to high counts.

Over 60 sheep carcasses that had been held without evisceration for 24 h at 20°C were examined. Eighteen of these were exercised to exhaustion before slaughter, as post mortem invasion was supposedly enhanced in carcasses from exhausted animals. In no case was there any indication that the muscle tissue was other than sterile. The obvious inference about post mortem invasion was confirmed by experiments with mice and guinea-pigs, with which it was shown that there was no movement of bacteria from the intestine until the stomach ruptured some 2 to 3 days after death, releasing bacteria into the abdominal cavity. After this all tis-

sues were rapidly invaded (Gill, Penney & Nottingham, 1979).

The ease with which the non-occurrence of post mortem invasion can be demonstrated raises the question of why this unlikely hypothesis was previously accepted. The reason probably lies in the nature of the evidence which was considered, which consisted only of the apparent demonstration of bacteria in deep tissues without any indication of how they got there. The likelihood of spurious results is obvious when it is realized that a single contaminating bacterium would be reported as up to 100 bacteria per gram of tissue, and that with few exceptions most reports of bacteria in tissues make no mention of any attempt to evaluate the efficiency of the supposedly aseptic techniques used.

Perimortem Invasion

Another suggestion of 19th century pathology, termed "agonal" invasion, was that at death the intestine suddenly became permeable to bacteria. This hypothesis at least has the merit that bacteria reaching the blood stream could be transported throughout the body before the circulation ceased. There is, however, no evidence for the occurrence of agonal invasion, while in many cases it can be totally discounted, so the hypothesis is no more tenable than post mortem invasion.

There remains the possibility that bacteria can be introduced from without during the slaughtering process. By using slaughter instruments heavily contaminated with bacteria readily identified by genetic markers it has been shown that such contamination is possible (Mackey & Derrick, 1979).

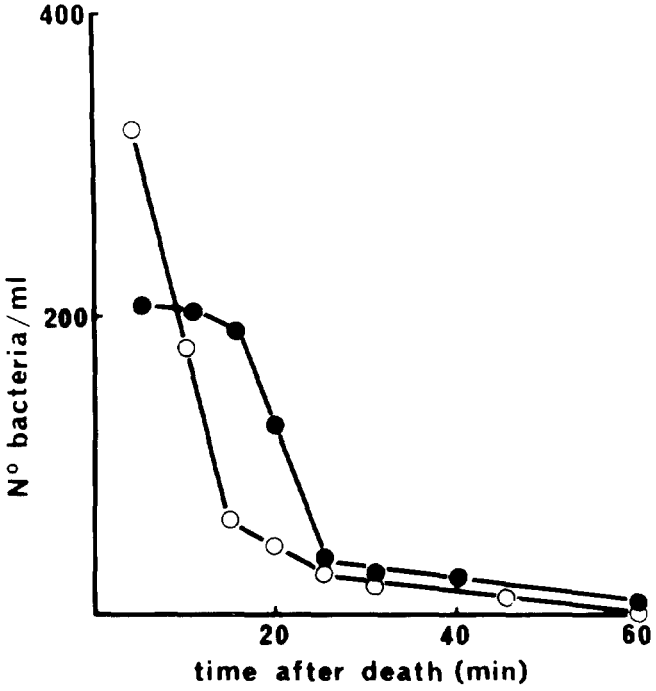

Figure 3. Bacteria recovered from blood of carcasses injected intravenously with bacterial suspension before death. *E. coli* (○); *C. perfringens* (●).

The hygienic importance of such contamination will depend upon the ability of bacteria to survive in the carcass. The bactericidal activities of blood and lymph are well known, so it would be expected that small numbers of bacteria introduced during slaughter could be entirely eliminated. Bacteria injected into the bloodstream of animals just before slaughter continue to be rapidly eliminated for the first 20 to 30 minutes after death (Fig. 3). Those that survive this initial period can commence growth 3 to 4 hours after death in carcasses held at 30°C. The maximum initial number of bacteria that can be totally eliminated varies with the species, *Clostridium perfringens* and *Salmonella* being markedly less susceptible to elimination than the other species examined (Table 4). Even so, with a minimum of 20 cells per gram of carcass an initial inoculum of 10^7 cells would be required to permanently contaminate a beef carcass, and something in excess of 10^5 cells for a sheep (Gill & Penney, 1979). Moreover, simultaneous entry of subviable numbers of other species does not seem to reduce the numbers of the relatively resistant species required for survival. It therefore seems that only a very dirty operation would permanently contaminate carcasses by this route, though with variation in both bactericidal activity between carcasses and resistance of bacterial strains, considerably lower numbers of bacteria might suffice in some cases.

Table 4. Maximum Initial Numbers of Bacteria Completely Eliminated from Carcass Tissues

	No. bacteria/g
P. aeruginosa	600
E. coli	1,000
S. aureus	500
C. perfringens	20
S. typhimurium	20

Antemortem Contamination

Bacterial invasion of the living animal occurs through lesions and undamaged mucosal surfaces. In healthy animals this invasion is countered by the immune defence mechanisms. If these are defective the animal is highly susceptible to infection by "opportunistic" bacteria (von Graevenitz, 1977). It is therefore possible to conceive of a dynamic bacterial population in the tissues of healthy animals which is continually renewed from without. However, all available evidence indicates that tissue fluids are normally sterile, so the rate of invasion of the healthy animal by bacteria must be very small in comparison with the body's ability to deal with them.

Diseased animals would obviously harbour bacteria. Those showing symptoms should be detected during ante- and post-mortem inspection, but some pathogenic bacteria may be present in tissues without any overt symptoms. *Salmonellae* are a particular problem in this respect. When present they would usually be found in organs or lymph nodes rather than muscle tissue (Ayres, 1978).

Finally, there is the question of bacterial spores in deep tissues. A number of studies have indicated the presence of

Clostridium perfringens at a level of 1 to 10 per gram in the liver, spleen and lymph nodes of a significant proportion of healthy animals. The only published work on spore contamination of living tissues concerns spores of *Clostridium novyi*, the causative agent of the animal disease, necrotic hepatitis. The oxygen tension in healthy tissue is too high to allow growth of clostridia, but infecting flukes produce necrotic tracts in the liver in which spores can germinate. The subsequent growth of the bacterium is accompanied by toxin production which causes the death of the animal (Jamieson, 1949).

Several hours after oral administration of a spore preparation, Bagadi & Sewell (1974) isolated *C. novyi* from sheep livers. Spores could not be found in the blood-stream after dosing, so the authors suggested that they travelled to the liver via the lymphatics. Spores were found to persist in the livers of otherwise healthy animals for several weeks after they were dosed. These results demonstrate the aetiology of necrotic hepatitis, but the numbers of spores reaching and surviving in the tissues were not reported. These details are important when considering the probabilities of deep tissue contamination by clostridial spores.

Cleaned spores of *C. perfringens* were injected intravenously into mice and the tissues subsequently examined for the presence of the bacterium. Spores were present in most tissues after 24 h with any inoculum between 10^3 and 10^6 spores per gram of body weight, but only in animals which had received 10^6 spores per gram of body weight were spores consistently found outside the liver after 48 h. With this dose most tissues were still contaminated, but with an increased proportion of spores in the liver. The number of spores in the liver declined during the next four weeks, but after this there was little decrease in numbers (Table 5). In a few cases spores also persisted in the spleen, but in smaller numbers than in the liver. Vegetative cells injected intravenously were eliminated from all tissues in a few hours. When spores were injected intraperitoneally, larger numbers were required to give contaminated tissue; even with 10^5/g no spores were detected after 24 h. With large numbers of spores, results were similar to those obtained with intravenous injection. Initial results indicate that spores of *C. novyi* and *C. septicum* behave in the same manner as those of *C. perfringens* when injected into mice. It appears that all spores will be eliminated within a week or two unless large inocula are given, when a small fraction of the spores survive in the liver for an indeterminate but lengthy period.

Table 5. Survival of Spores of *C. perfringens* in Mouse Livers After Intravenous Injection of 2×10^5 Spores/g Body Weight

Time (weeks)	*No. spores/g liver*
1	1.8×10^4
2	1.3×10^4
3	6.6×10^3
4	2.3×10^3
5	3.4×10^3
6	1.1×10^3
7	2.1×10^3

Attempts to obtain contaminated tissue by feeding spores in the drinking water or introducing spores directly into the stomach via a catheter gave consistently negative results. This contrasts with the reports of tissue contamination following feeding of *C. novyi* spores, and may have occurred because we were using preparations of cleaned spores, whereas the work with *C. novyi* used crude spore preparations. These crude preparations could have contained residual toxins or other materials which damaged the intestinal wall, allowing spores to enter the blood-stream. Uptake via the lymphatics seems unlikely, as spore survival following intraperitoneal injection suggests that spores entering the lymphatics are less likely to survive than those entering the blood-stream.

It would seem that deep tissues are only likely to be contaminated by specific organisms which have reached the tissues during the life of the animal. The organisms involved will be pathogens, particularly Salmonellae, or clostridial spores. They will usually be localized in the viscera and associated lymph nodes rather than muscle tissue, so even when meat cuts are held at high ambient temperatures, spoilage due to growth of intrinsic bacteria must be a rare phenomenon.

For meat hygiene it is important to know the conditions under which any intrinsic organisms could grow and spread from their initial sites in the carcass. In experimentally contaminated meat held at 30°C, slow growth of vegetative cells is initiated 2 to 3 hours after death, and of spores 4 to 5 hours later (Gill, Penney & Nottingham, 1979). The behaviour of deep tissue contaminants in the state in which they would usually be found in carcasses does not seem to have been studied.

Conclusions

There are still important areas in meat microbiology that are poorly understood. In some cases misunderstanding is not due to a paucity of facts, but seems to arise because of the existence of a large body of data obtained under poorly defined practical conditions. Certainly meat microbiology is an applied science, but it is far easier to understand particular cases if the basic principles are clearly established. On this consideration alone some fundamental studies are necessary, but there is another area where they can be of prime importance. Hygiene regulations for the meat industry are largely derived from consideration of general principles, and if the understanding of these is incomplete or erroneous, some regulations are likely to be ineffective or even deleterious. Because of the associated costs, it is becoming increasingly obvious that regulations cannot be indefinitely proliferated, however laudable their intentions. Those responsible for public health and safety must, however, act to control any situation which is seen as a threat to the consumer, and it is all too easy to deduce the worst from indirect and incomplete evidence. The only solution is a good understanding of the system that the regulations are designed to control.

The problem of intrinsic bacteria would seem to be a cautionary example of what can occur. The organisms must reach the tissues during the life of the animal, so the number

of animals carrying any intrinsic flora will be highly variable and nothing can be done after the animals are dead to reduce the number affected. Postmortem hygiene regulations should therefore be concerned with preventing growth of intrinsic bacteria, but current regulations are concerned with circumventing the mythical invasion from the intestine. Evisceration must be carried out soon after death to remove the source of corruption, bruises must be removed to ensure that the bacteria within them do not spread to healthy tissues, and bleeding-out must be complete to prevent bacterial invasion. While some of these practices may help cooling of the carcass, none of them is necessary for reasons of hygiene, and all must give rise to unnecessary loss of perfectly sound animal protein. There are also proposals that a regulation be introduced specifying the time within which a deep tissue temperature of 7°C must be attained. Clostridia, when present, would be largely confined to the liver and spleen, and would not grow in meat below 20°C anyway. This regulation must therefore be mainly concerned with preventing growth of salmonellae. However, there are no data on the time required after death for growth of salmonellae originally present in carrier state to be initiated and for the organisms to spread from the sites of infection in organs and lymph nodes. The details of the time-temperature relationship will therefore be an informed guestimate on the basis of growth rates of salmonellae on meat and the time necessary to cool a large carcass. Such a regulation may therefore still be either ineffective or too severe. But even if it does achieve its purpose, it will add to and not supercede existing regulations.

It is clear that there must be regulation in the meat industry and that it will increase. New regulations will be based on the available data, and if facts are lacking it is quite probable that some will be based on fallacies. The only sure specific against such an eventuality is a sound understanding of basic bacterial behaviour with respect to meat. Meat microbiology is an applied science and as such must be concerned with practical results. Unfortunately the accumulation of data from poorly defined systems can sometimes confuse rather than resolve problems, so there is a real need for more fundamental study if attractive but erroneous hypotheses are not to give rise to ineffective practices which neither the industry nor the consumer can afford.

References

Ayres, J. C. 1978. Salmonella in meat products. pp. 148-155 Proc. 31st Ann. Recip. Meat Conf. Am. Meat Sci. Assn. Storrs, Connecticut.

Bagadi, H. O. & Sewell, M. M. H. 1974. A study of the route of dissemination of orally administered spores of *Clostridium novyi* type B in guinea pigs and sheep. Res. Vet. Sci. 17:179-181.

Buckley, J., Morrissey, P. A. & Daly, M. 1976. Aseptic technique for obtaining sterile beef tissue. J. Food Technol. 11:427-430.

Canada, J. C. & Strong, D. H. 1964. *Clostridium perfringens* in bovine livers. J. Food Sci. 29:862-864.

Gill, C. O. 1976. Substrate limitation of bacterial growth at meat surfaces. J. Appl. Bacteriol. 41:401-410.

Gill, C. O. 1979. Intrinsic bacteria in meat. J. Appl. Bacteriol. 47:367-378.

Gill, C. O. & Newton, K. G. 1977. The development of aerobic spoilage flora on meat stored at chill temperatures. J. Appl. Bacteriol. 43:189-195.

Gill, C. O. & Newton, K. G. 1979. Spoilage of vacuum-packaged dark, firm, dry (DFD) meat at chill temperatures. Appl. Environ. Microbiol. 37:362-364.

Gill, C. O. & Penney, N. 1977. Penetration of bacteria into meat. Appl. Environ. Microbiol. 33:1284-1286.

Gill, C. O. & Penney, N. 1979. Survival of bacteria in carcasses. Appl. Environ. Microbiol. 37:667-669.

Gill, C. O., Penney, N. & Nottingham, P. M. 1979. Tissue sterility in uneviscerated carcasses. Appl. Environ. Microbiol. 36:356-359.

Glenn, A. R. 1976. Production of extracellular proteins by bacteria. Ann. Rev. Microbiol. 30:41-62.

Ingram, M. & Dainty, R. H. 1971. Changes caused by microbes in spoilage of meat. J. Appl. Bacteriol. 34:21-39.

Jamieson, S. 1949. The identification of *Clostridium oedematiens* and an experimental investigation of its role in the pathogenesis of infectious necrotic hepatitis (black disease) of sheep. J. Pathol. Bacteriol. 61:389-402.

Lepovetsky, B. C., Weiser, H. H. & Deatherage, F. E. 1953. A microbiological study of lymph nodes, bone marrow and muscle tissue obtained from slaughtered cattle. Appl. Microbiol. 1:57-59.

Mackey, B. M. & Derrick, C. M. 1979. Contamination of the deep tissues of carcasses by bacteria present on the slaughter instruments or in the gut. J. Appl. Bacteriol. 46:355-366.

Moran, N., Torres-Anjel, M. J., Wiggins, A. & Riemann, H. P. 1973. Prevaluncia de *Clostridium perfringens* incluyendo cepas potencialmente patogenas paru el hombre, en ganado bovino de carne. Proc. 7th Panam. Cong. Vet. Med. Animal Husband., Bogota.

Narayan, K. G. 1966. Studies on the clostridia incidence in beef cattle. Act. Vet. Acad. Sci. Hungar. 16:65-72.

Newton, K. G. & Gill, C. O. 1978. The development of the anaerobic spoilage flora of meat stored at chill temperatures. J. Appl. Bacteriol. 44:91-95.

Newton, K. G. & Rigg, W. J. 1979. The effect of film permeability on the storage life and microbiology of vacuum packed meat. J. Appl. Bacteriol. 47:433-441.

Ockerman, H. W., Cahill, V. R., Weiser, H. H., Davis, C. E. & Siefker, J. R. 1969. Comparison of sterile and inoculated beef tissue. J. Food Sci. 34:93-99.

Radouco-Thomas, C., Lataste-Dorolle, C., Zender, R., Busset, R., Meyer, H. M. & Mouton, R. F. 1959. The anti-autolytic effect of epinephrine in skeletal muscle: non-additive process for preservation of meat. Food Res. 24:453-482.

Smith, L. D. & Jasmin, A. M. 1956. The recovery of *Clostridium hemolyticum* from the livers and kidneys of apparently healthy cattle. Am. Vet. Med. Assn. J. 129:68-71.

Tarrant, P. J. V., Pearson, A. M., Price, J. F. & Lechowich, R. V. 1971. Action of *Pseudomonas fragi* on the proteins of pig muscle. Appl. Microbiol. 22:224-228.

Vanderzant, C. & Nickelson, R. 1969. A microbiological examination of muscle tissue of beef, pork and lamb. J. Milk Food Technol. 32:357-361.

Von Graevenitz, A. 1977. The role of opportunistic bacteria in human disease. Ann. Rev. Microbiol. 31:447-471.